J-TEC

LabCyte

Application guide

Content

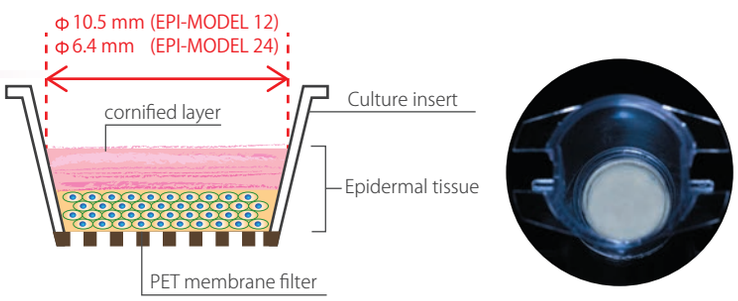
3-D cultured human epidermis

LabCyte EPI-MODEL

LabCyte EPI-MODEL is a 3-D human cultured epidermis produced from normal human epidermal cells (keratinocytes) that are cultured at the air-liquid interface to become stratified. LabCyte EPI-MODEL was developed as an alternative to animal experimentation performed in skin irritation tests. Because it is composed of human epidermal cells, the structure of LabCyte EPI-MODEL is morphologically similar to that of the human epidermis. Furthermore, LabCyte EPI-MODEL has a high in vivo-in vitro correlation, and very low lot-to-lot variability. LabCyte EPI-MODEL can be used to determine the skin irritation potential of a wide range of chemicals through the analysis of cell viability using the MTT assay. Moreover, since LabCyte EPI-MODEL is metabolically active, it can also be used to evaluate biological processes, such as the production of cytokines and growth factors, in response to different test substances.

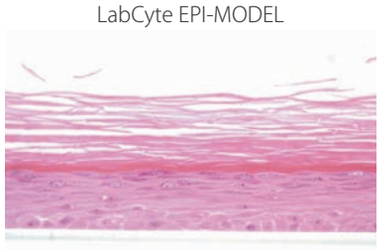
A cultured model that closely reproduces human epidermal features

Model structure

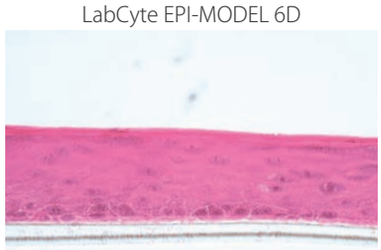


Tissue structure

LabCyte EPI-MODEL



LabCyte EPI-MODEL 6D



Normal human epidermis (forearm)

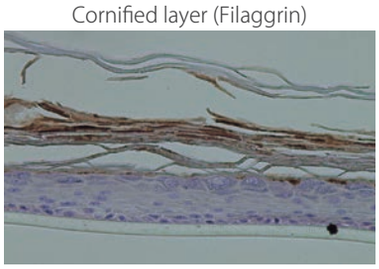
Courtesy of Dr. Y. Kitajima (Gifu Univ.)

EPI-MODEL has a structure similar to that of the epidermis, with a basal layer, a spinal layer, a granulous layer, and a cornified layer.
EPI-MODEL 6D is a cultured epidermal model with an under-developed cornified layer.

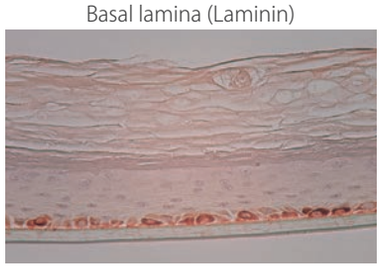
Protein expression pattern analysis

The expression pattern of proteins specifically expressed at different layers of the epidermis

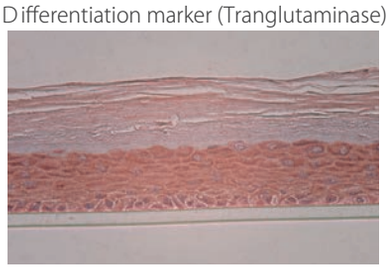
Cornified layer (Filaggrin)



Basal lamina (Laminin)



Differentiation marker (Tranglutaminase)



Customer support

Japan Tissue Engineering Co. Ltd is responsible for the development, manufacture and sales of LabCyte products, and is able to offer broad support to all customers through our highly trained technical specialists.

Skin Irritation Test and other applications

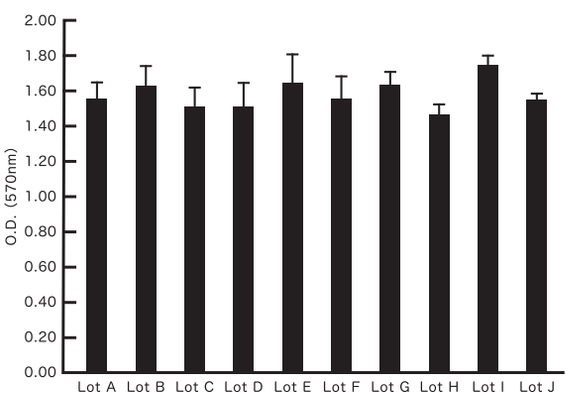
LabCyte EPI-MODEL is an alternative to animal experimentation in skin irritation and toxicity tests. It can be used in pharmaceutical, dermatological and basic research. The EPI-MODEL 6D, a 3D epidermal model with under-developed cornified layers, can be used in the risk assessment of mild irritants, and the analysis of ceramide production in vitro.

Production of highly reproducible models using gold standard techniques

LabCyte EPI-MODEL is produced by highly trained and specialized staff. We guarantee the delivery of high quality products by implementing strict in house quality control tests.

*EPI-MODEL data (taken in house)

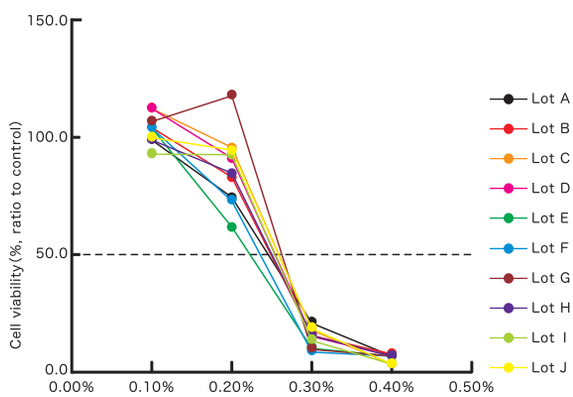
Live cell count (within-lot, lot-to-lot variation)



	Lot A	Lot B	Lot C	Lot D	Lot E	Lot F	Lot G	Lot H	Lot I	Lot J
平均	1.56	1.63	1.51	1.51	1.65	1.56	1.64	1.47	1.75	1.55
SD	0.09	0.12	0.11	0.15	0.16	0.12	0.08	0.06	0.05	0.04
CV(%)	5.8	7.4	7.1	9.8	9.7	7.9	4.6	4.2	3.0	2.9

Data demonstrating the low within-lot and lot-to-lot variation in cell viability.

Barrier function analysis (SLS 18 h exposure)

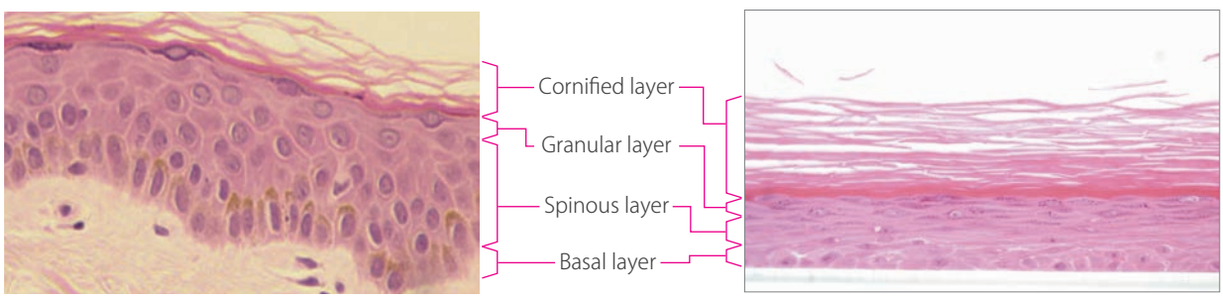


	Lot A	Lot B	Lot C	Lot D	Lot E	Lot F	Lot G	Lot H	Lot I	Lot J
IC50	0.25	0.25	0.26	0.25	0.22	0.24	0.26	0.25	0.25	0.26

Data demonstrating the high reproducibility of results between different lots.

Tissue structure

EPI-MODEL has a structure similar to that of the epidermis,
comprising of a basal layer, a spinal layer, a granulous layer, and a cornified layer.

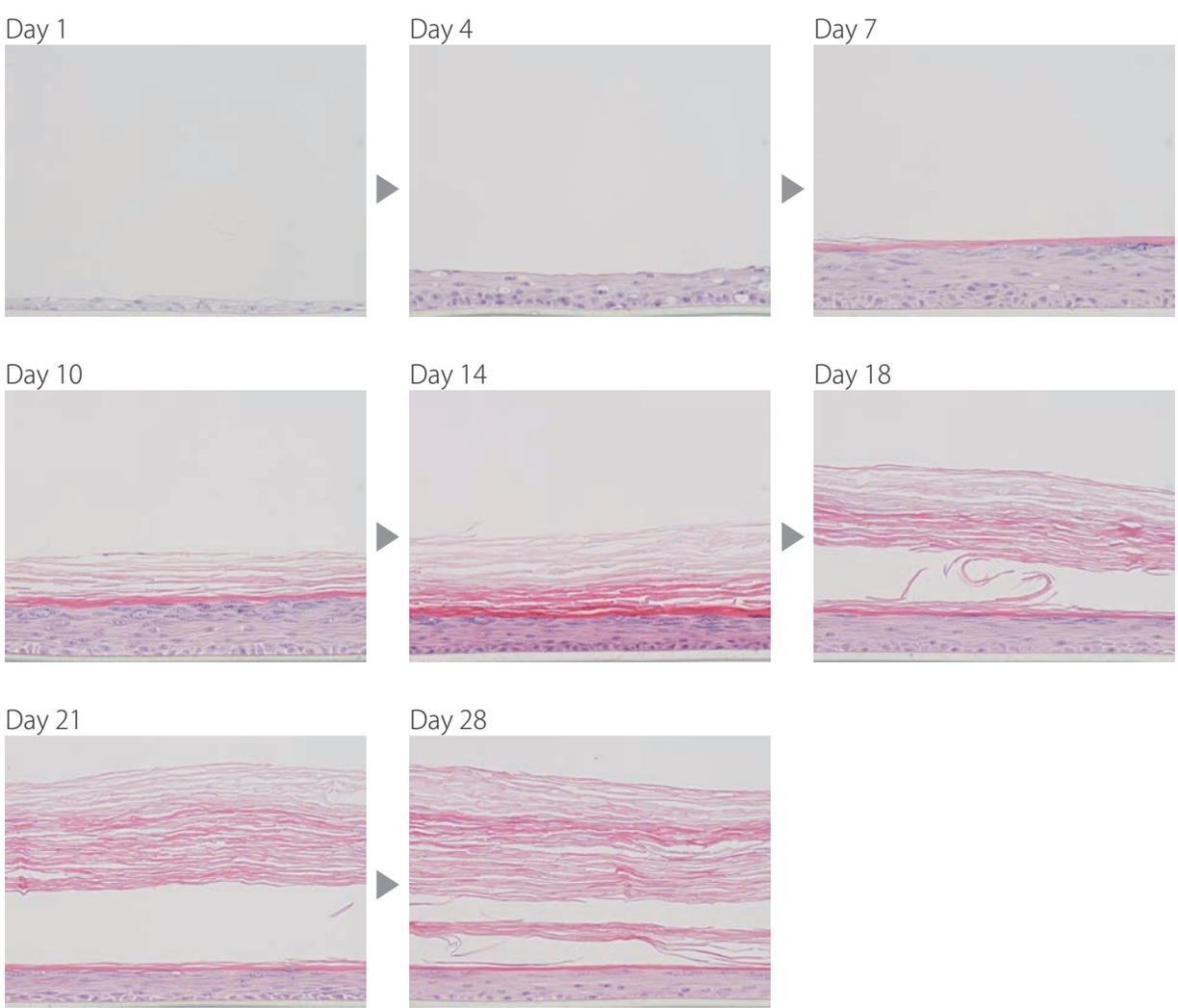


Normal human epidermis (forearm)

LabCyte EPI-MODEL

Courtesy of Dr. Y. Kitajima (Gifu Univ.)

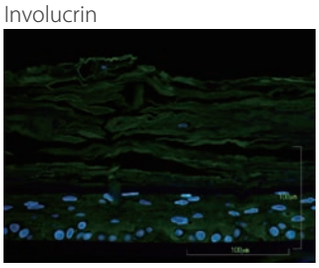
Morphological and structural changes of the different epidermal layers during culture.



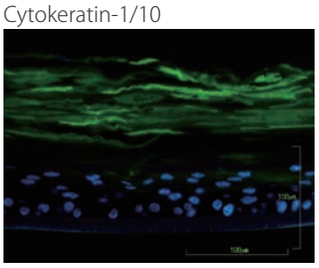
Immunohistochemistry

Detection of proteins expressed in the cornified and viable layers of the epidermis.

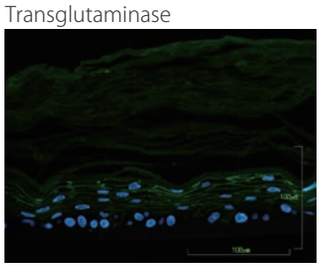
Involucrin



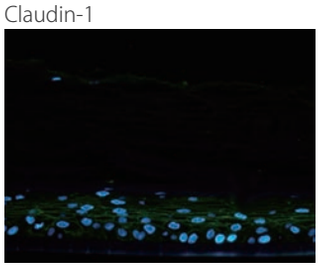
Cytokeratin-1/10



Transglutaminase



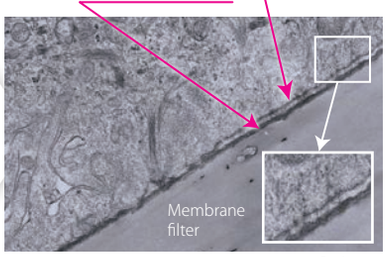
Claudin-1



Ultrastructure

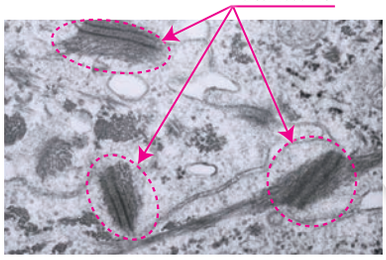
Ultrastructure of epidermal layers observed by transmission electron microscopy

Basal Layer

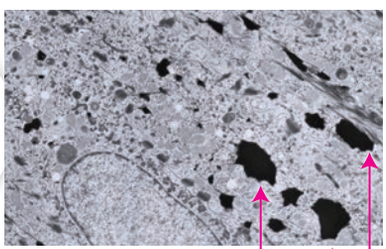


Magnified area:
presence of immature hemidesmosomes

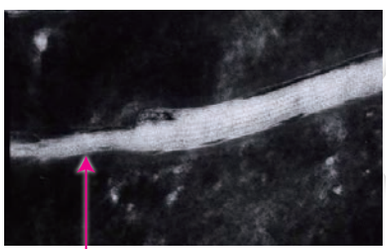
Spinous Layer



Granular Layer

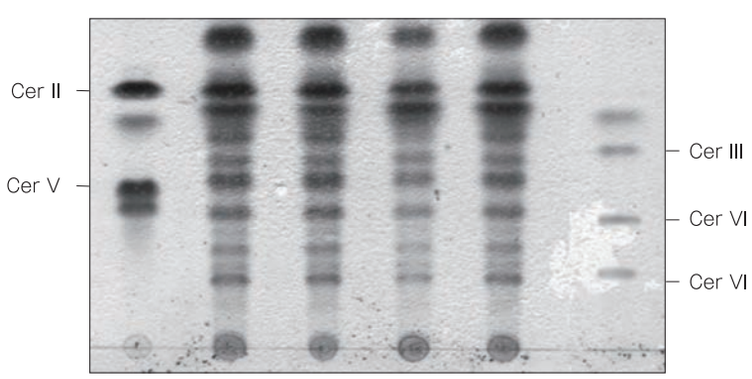


Cornified Layer



Ceramide analysis

EPI-MODEL produces high levels of ceramide



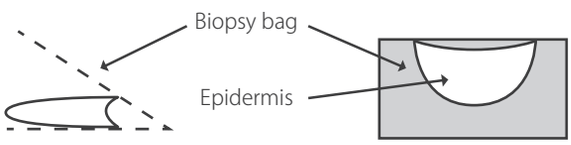
Courtesy of Dr. Y. Tokudome (Josai Univ.)
Skin Pharmacol Physiol 2011;24:218-223

Protein expression analysis by immunohistochemistry

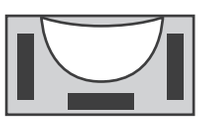
Protocol

Paraffin embedding

① Transfer the culture insert containing the EPI-MODEL to a tube with 4% Paraformadelhyde (PFA).
② Leave it overnight at 4℃ to fix the epidermal tissue.
③ Cut the PET membrane off from the culture insert using a scalpel.
④ Transfer enough 4% agar solution to fill half of a well of a 6-well plate.
⑤ Transfer the epidermal tissue to the well before the agar completely solidifies.
⑥ Top the well with 4% agar solution and wait until it completely solidifies.
⑦ Trim the excess of agar with a scalpel.
⑧ Transfer the tissue embedded in agar to a biopsy bag.



⑨ Close the biopsy bag surrounding the tissue using a stapler.



⑩ Sandwich the biopsy bag in between a folded Tissue-Tek foam biopsy sheet.
⑪ Put the tissue in paraffin embedding cassettes.
⑫ Rinse the tissue in tap water.
⑬ Remove the excess of water from the cassette and proceed with the paraffin embedding protocol.

Paraffin embedding program

Perform this step according to the instructions of the equipment being used in your laboratory.

① 70% ethanol	60 min
② 100% ethanol	90 min × 5 cycles
③ Xylene	60 min × 4 cycles
④ Paraffin (63℃)	45 min × 4 cycles

Preparation of tissue sections

① Prepare paraffin blocks according to the instructions of the equipment being used in your laboratory.
② Store the paraffin blocks at 4℃.
③ Cut 3 mm-thick tissue sections.
④ Mount sections on glass slides.
⑤ Dry the slides at 40 - 60℃ overnight.

Immunohistochemistry

Deparaffinization

① Incubate slides in xylene three times for 2 min each.
② Incubate slides in 100% ethanol three times for 1 min each.
③ Wash slides in pure water for 1 min.
④ Leave slides in pure water.

Antigen retrieval (choose according to the antibody being used)

❶ Heat-induced antigen retrieval

① Put slides in a container with the buffer of your choice:
- For pH 6: Target Retrieval Solution, Citrate pH 6（Dako）
- For pH 9: Target Retrieval Solution, Tris-EDTA pH 9（Dako）

② Bring slides to 95℃ and incubate them according to the antibody being used.
③ After incubation, leave the slides in the buffer to cool down at room temperature.
④ Transfer the slides to PBS.
⑤ Circle the tissue sections with a PAP pen.

❷ Proteinase K-induced antigen retrieval

① Put slides in a container with PBS.
② Circle the tissue sections with a PAP pen.
③ Transfer the slides to a humidified chamber.
④ Add Proteinase-K solution (Dako) to the tissue sections.
⑤ Incubate at room temperature.
⑥ Wash slides in DPBS-T three times for 2 min each.

Endogenous peroxidase blocking

① Incubate slides in 0.3% hydrogen peroxide for 30 min at room temperature.
② Wash slides in DPBS-T three times for 2 min each.

Blocking

① Transfer the slides to a humidified chamber.
② Add 100 µl of Blocking One (Nacalai Tesque) to each slide.
③ Block slides for at least 30 min at room temperature.

Primary antibody

① Dilute the primary antibody with Dako REAL antibody diluent (Dako).
② Remove the blocking solution and add 100 µl of the diluted antibody solution to each slide.
③ Incubate for 1 h at room temperature.

Secondary antibody

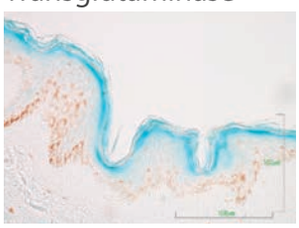
Secondary antibody solution:
・Anti-rabbit secondary antibody (HRP-conjugated)
ImmPRESS Reagent, Anti-Rabbit Ig（VECTOR）
・Anti-mouse secondary antibody (HRP-conjugated)
ImmPRESS Reagent, Anti-Mouse Ig（VECTOR）
① Wash slides in DPBS-T three times for 2 min each.
② Add 100 µl of the appropriate secondary antibody solution.
③ Incubate for 30 min at room temperature.
④ Wash slides in DPBS-T three times for 2 min each.
⑤ Wash slides in DPBS for 2 min.
Signal staining using Histogreen (AbCys)
⑥ Add 2 drops of Histogreen-Chromogen (No. 1) to 1 ml of Histogreen-Buffer (No. 2) and mix well.
⑦ Prepare HistoGreen by adding two drops of H_2O_2 (No. 3) to the solution prepared above. Mix well.
⑧ Add 100 µl of HistoGreen to each slide.
⑨ Incubate for 1 - 5 min at room temperature.
⑩ Wash slides in DPBS three times for 2 min each.
⑪ Shortly wash in pure water.

Dehydration and mounting

① Incubate slides in 100% ethanol three times for 30 sec each.
② Incubate slides in ethanol : xylene (1:1) mixture for 30 sec.
③ Incubate slides in xylene three times for 30 sec each.
④ Mount sections with coverslips using VectaMount™ Permanent Mounting Medium (Vector)

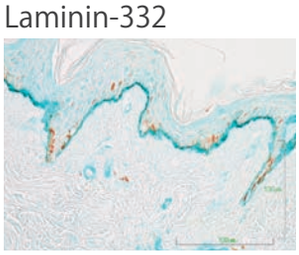
Human skin	EPI-MODEL24		
	Day 0	Day 3	Day 7

Transglutaminase



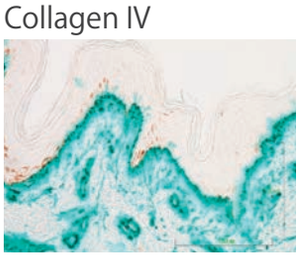

Expression detected from spinous to cornified layer.

Laminin-332




Expression detected in the basal and spinous layers, as culture progressed.

Collagen IV



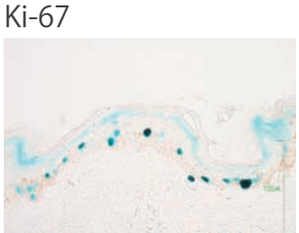

Expression detected in the basal layer.

Collagen VII




Expression detected in the basal layer.

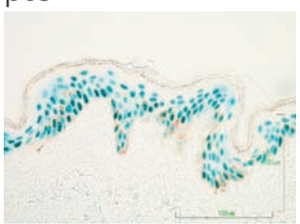
Ki-67

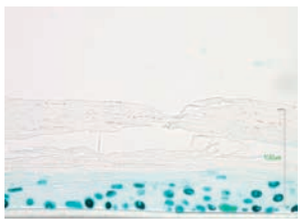



Ki67 is a widely used proliferation marker.
Expression detected in the cells of the basal layer, as it is observed in human skin.

Human skin	EPI-MODEL24		
	Day 0	Day 3	Day 7

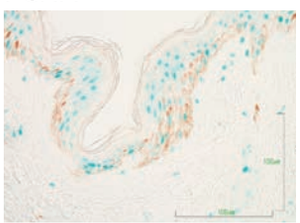
p63

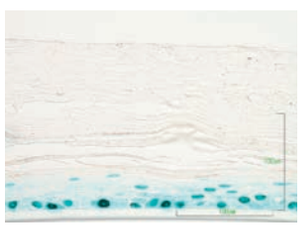




p63 is often referred to as a marker for epidermal stem cells.
Expression detected in the basal and spinous layers, as it is described in human skin.

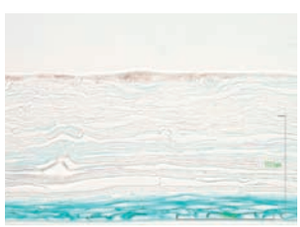
PCNA





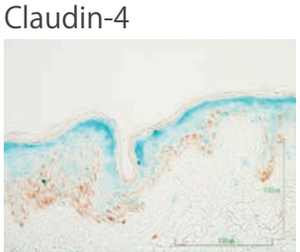
PCNA is a widely used proliferation marker.
Expression detected in the cells of the basal layer, as it is described in human skin.

Claudin-1



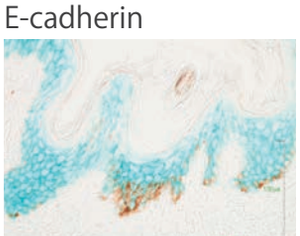
Claudin-1 is a tight junction marker.
Expression detected from basal to granular layer, similar to human skin.

Claudin-4



Claudin-4 is a tight junction marker.
Expression detected from basal to granular layer, similar to human skin.

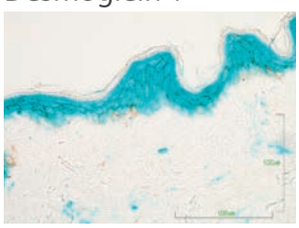
E-cadherin



E-cadherin is an adherens junction marker.
Expression detected from basal to granular layer, similar to human skin.

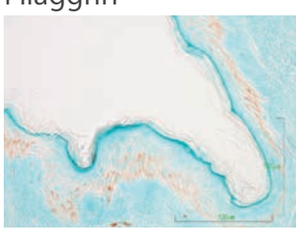
Human skin	EPI-MODEL24		
	Day 0	Day 3	Day 7

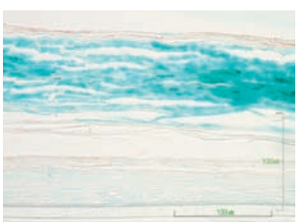
Desmoglein 1



Desmoglein is a desmosome marker.
Expression detected between the basal and granular layer, similar to human skin.

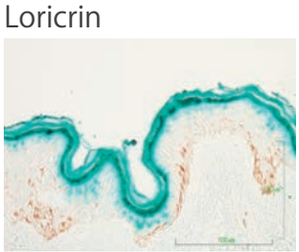
Filaggrin





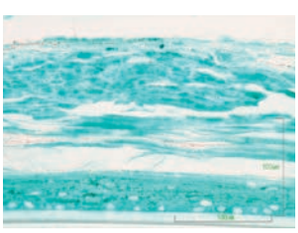
Expression detected from the granular to cornified layer, similar to human skin.

Loricrin



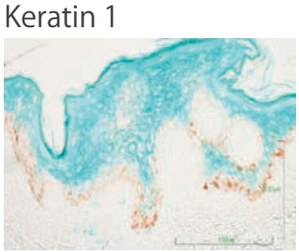
Expression detected in the granular layer, similar to human skin.

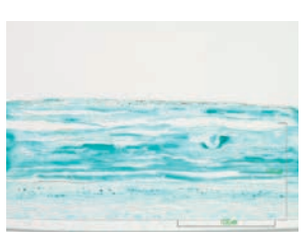
Involucrin



Involucrin is an epidermal differrentiation marker.
Unlike in human skin, expression was detected in all layers of the epidermal tissue.

Keratin 1

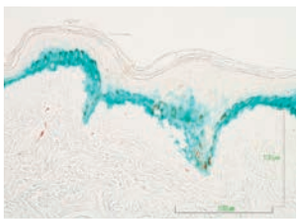




Keratin 1 is an epidermal differentation marker.
Expression detected from spinous to cornified layer, similar to human skin.

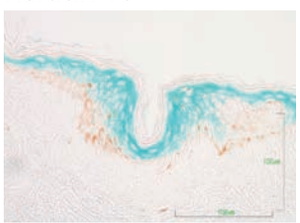
Human skin	EPI-MODEL24		
	Day 0	Day 3	Day 7

Keratin 5



Keratin 5 is a basal layer marker.
Expression detected in the basal layer, similar to human skin.

Keratin 10



Keratin 10 is an epidermal differentation marker.
Expression detected from spinous to cornified layer, similar to human skin.

Keratin 14

Keratin 14 is a basal and spinous layer marker.
Expression detected in the basal and spinous layers, similar to human skin.

Protein expression analysis by Western blotting

Protein extraction

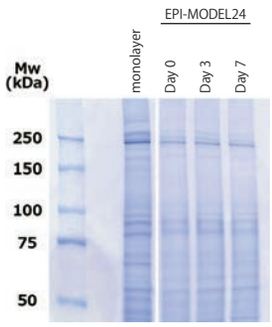
① Rinse the epidermal tissue with PBS and cut the membrane off from the culture insert with a scalpel.
② Transfer the tissue to a 1.5 ml microtube.
③ Leave the microtube on ice. If necessary, store the tube at -80℃, and thaw the tissue on ice prior use.
④ Transfer the tissue to a 10 cm dish, and cut it in small pieces with a scalpel.
⑤ Transfer the tissue fragments to a new 1.5 ml microtube.
⑥ Add 200 - 300 µl of RIPA buffer to each microtube.
⑦ Homogenize the tissue using a BioMasher®II (Nippi).
⑧ Sonicate for 30 min (37 W).
⑨ Centrifuge the microtube for 10 min (15,000 x g, 4℃).
⑩ Transfer the supernatant to a new 1.5 ml microtube.
⑪ Centrifuge the microtube for 10 min (15,000 x g, 4℃).
⑫ Transfer the supernatant to a new 1.5 ml microtube.
⑬ Estimate the protein concentration by BCA or Bradford assay.

Immunoblotting

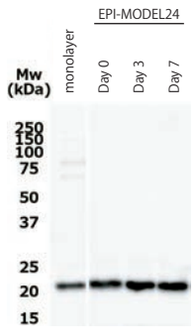
① Mix 10 µg of protein sample to 2x Laemmli buffer (SIGMA-ALDRICH) and mix well.
② Heat the sample to 100℃ for 2 min.
③ Briefly centrifuge the tube.
④ Load onto a SDS-PAGE gel (1 mm gel, Tris-Glycine, 15 mA).
⑤ Rinse the gel in Tris-Glycine buffer for 10 min.
⑥ Prepare a PVDF membrane (Amersham Hybond P PVDF 0.45) by incubating it in methanol for 1 min, and washing it in distilled water for 5 min.
⑦ After transfer, block the membrane in 5% skim milk in TBS-T (1 h at room temperature).
⑧ Dilute the primary antibody in 5% skim milk in TBS-T.
⑨ Transfer the membrane to the primary antibody solution and incubate overnight at 4℃.
⑩ Wash the membrane in 5% skim milk in TBS-T three times for 15 min each.
⑪ Wash the membrane in TBS-T for 5 min.
⑫ Dilute the secondary antibody in TBS-T.
⑬ Transfer the membrane to the secondary antibody solution and incubate for 2 h at room temperature.
⑭ Wash the membrane in TBS-T three times for 15 min each.
⑮ Treat the membrane with ECL Western Blotting Detection Reagents (GE Healthcare).
⑯ Reveal the signal using a LAS4000 or equivalent.

EPI-MODEL

CBB staining

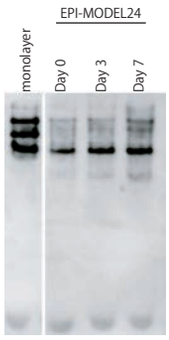


Claudin-1

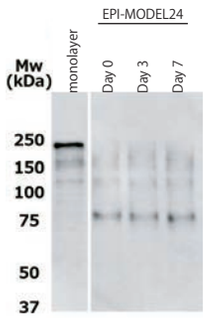


Results indicate the presence of tight junctions

Collagen IV

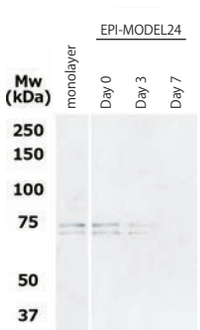


Native-PAGE



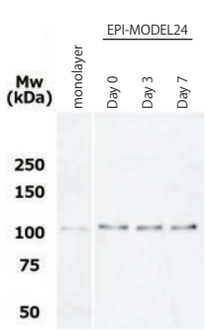
Results indicate the production of collagen IV

Collagen XVII



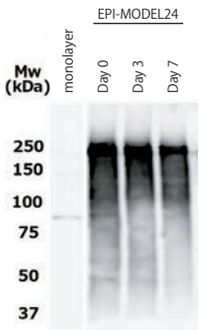
Results indicate the production of collagen XVII

E-Cadherin



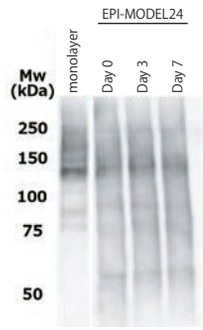
Results indicate the presence of adherens junction

Filaggrin



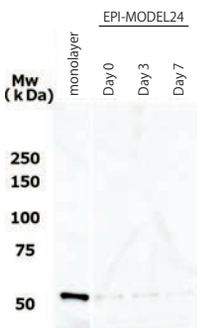
Results demonstrate the maturation of the cornified layer.

Involucrin



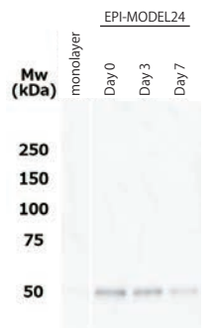
Results demonstrate the maturation of the cornified layer.

Keratin 5



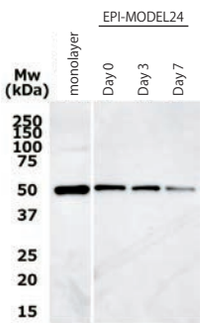
Results demonstrate the maintenance of the basal layer during culture.

Keratin 10



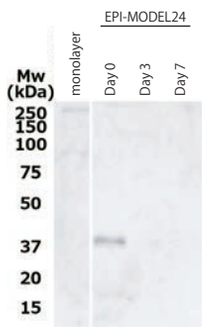
Results demonstrate the differentiation process induced by the 3D culture of epidermal cells.

Keratin 14



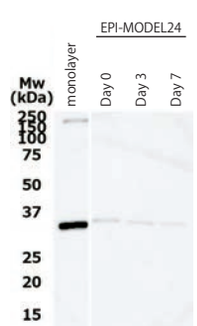
Results demonstrate the maintenance of the basal layer during culture.

Loricrin



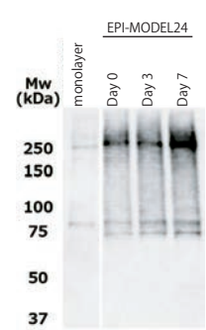
Results demonstrate the differentiation process induced by the 3D culture of epidermal cells.

PCNA



Results demonstrate the decrease of proliferating cells during culture.

Transglutaminase



Results demonstrate the maturation of the cornified layer.

Gene expression analysis by qPCR

RNA extraction

① Rinse the epidermal tissue with PBS.
② Cut the membrane off from the culture insert with a scalpel, and transfer the tissue to a 1.5 ml microtube.
③ Leave the microtube on ice. If necessary, store the tube at -80℃, and thaw the tissue on ice prior use.
④ Transfer the tissue to a 10 cm dish, and cut it in small pieces with a scalpel.
⑤ Transfer the tissue fragments to a new 1.5 ml microtube.
⑥ Extract the RNA using PureLink® RNA Mini Kit (Thermo Fisher).
⑦ Add 200 µl of lysis buffer to each tube.
⑧ Homogenize the tissue using a BioMasher®II (Nippi).
⑨ Using a seringe, pass the tissue fragments through a 21G needle at least 10 times.
⑩ Centrifuge the microtube at 2 min (12,000 x g, room temperature).
⑪Transfer the supernatant to a fresh microtube, add the same volume of 70% ethanol and mix well by vortexing.
⑫ Transfer 700 µl of the solution above to a spin column.
⑬ Centrifuge the microtube for 15 sec (12,000 x g, room temperature) and discard the flow through.
⑭ Repeat steps 12 and 13 if necessary.
⑮ Add 350 µl of Wash buffer I.
⑯ Discard the flow through and transfer the column to a fresh microtube.
⑰ Add 80 µl of PureLink DNA mixture.
⑱ Incubate for 15 min at room temperature.
⑲ Add 350 µl of Wash buffer I.
⑳ Centrifuge the microtube for 15 sec (12,000 x g, room temperature) and discard the flow through.
㉑ Add 500 µl of Wash buffer II.
㉒ Centrifuge the microtube for 15 sec (12,000 x g, room temperature) and discard the flow through.
㉓ Add 500 µl of Wash buffer II.
㉔ Centrifuge the microtube for 15 sec (12,000 x g, room temperature) and discard the flow through.
㉕ Centrifuge the microtube for 1 min (12,000 x g, room temperature) and discard the flow through.
㉖ Transfer the column to a fresh microtube.
㉗ Add 50 µl of Rnase-free water to the center of the filter of the spin column.
㉘ Incubate for 1 min at room temperature.
㉙ Centrifuge the microtube for 2 min (12,000 x g, room temperature).
The flow through contains the extraced RNA.
㉚ Use the Quant-iT™ RNA Assay Kit (Thermo Fisher) to estimate the RNA amount.

Reverse transcription and qPCR

① Transfer 100 ng of RNA to a PCR tube.

② Perform a reverse transcription reaction using the SuperScript® VILO™ cDNA Synthesis Kit (Thermo Fisher).

Temperature	Time
25℃	10 min
42℃	60 min
85℃	5 min

③ Dilute and aliquot the cDNA if necessary.

④ Perform qPCR using 5 - 10 ng of cDNA and Power SYBR® Green PCR Master Mix (Thermo Fisher).

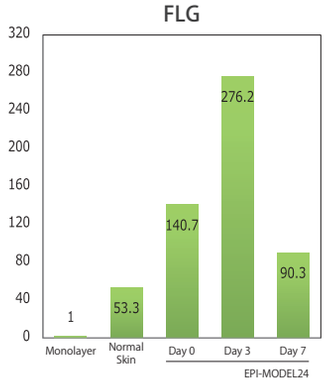
Temperature	Time	Number of cycles
95℃	10 min	1 cycle
94℃	15 sec	40 cycles
60℃	1 min	

Primers were designed using the following ProbeLibrary.

https://qpcr.probefinder.com/input.jsp?organism=h_sap

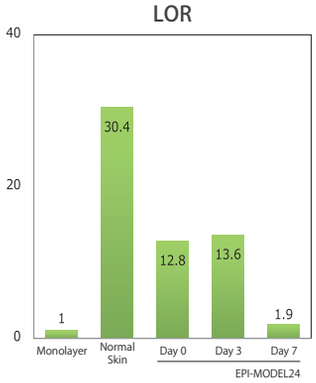
Fold of Control

FLG



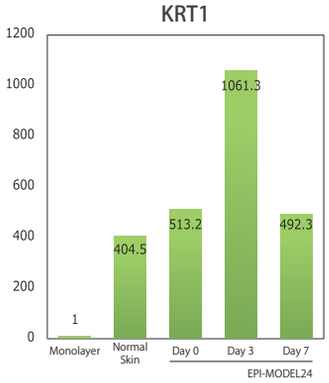
Results demonstrate the maturation of the cornified layer.

LOR



Results demonstrate the maturation of the cornified layer.

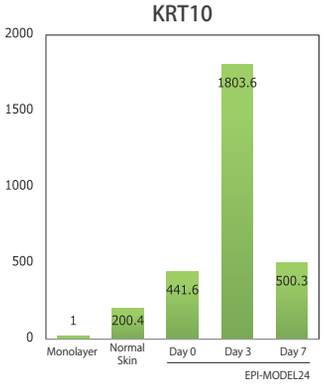
KRT1



Results demonstrate the maturation of the cornified layer.

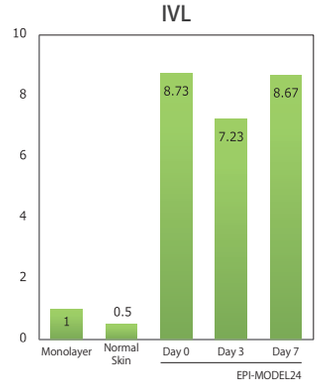
Fold of Control

KRT10



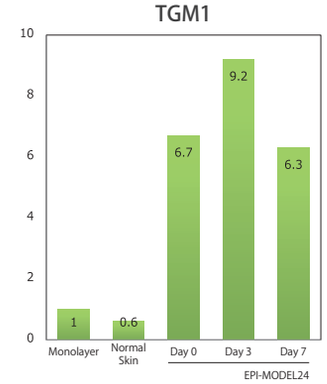
Results demonstrate the maturation of the cornified layer.

IVL



Results demonstrate the maturation of the cornified layer.

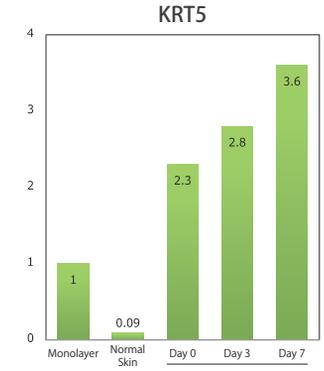
TGM1



Results demonstrate the maturation of the cornified layer.

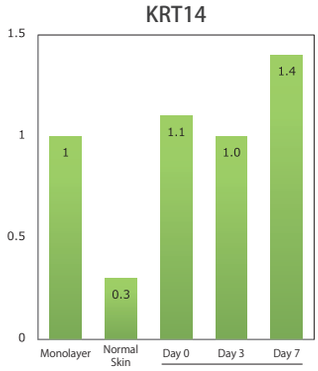
Fold of Control

KRT5



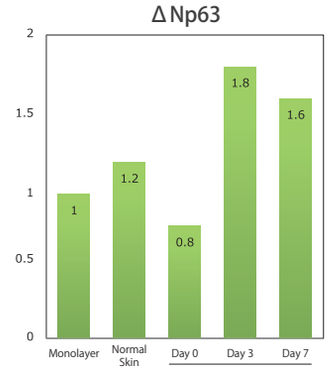
Results demonstrate the maintenance of the basal layer during culture.

KRT14



Results demonstrate the maintenance of the basal layer during culture.

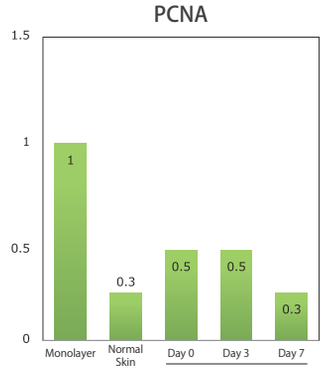
ΔNp63



Results demonstrate the maintenance of epidermal stem cells during culture.

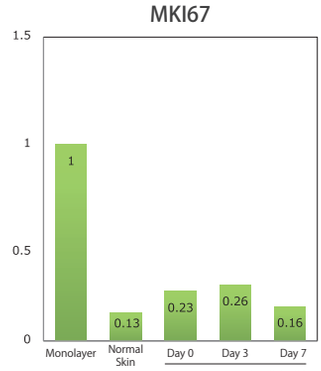
Fold of Control

PCNA



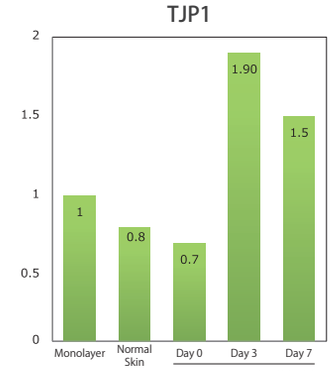
Results demonstrate the decrease of proliferating cells during culture.

MKI67



Results demonstrate the decrease of proliferating cells during culture.

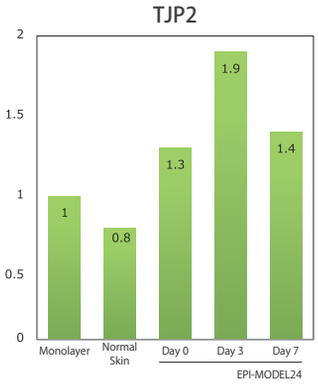
TJP1



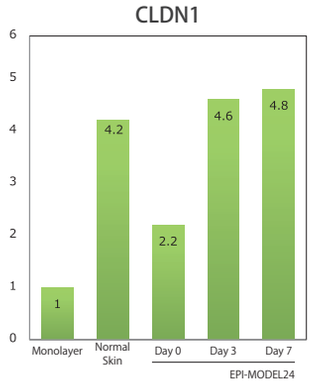
Results demonstrate the formation of tight junctions during culture.

EPI-MODEL

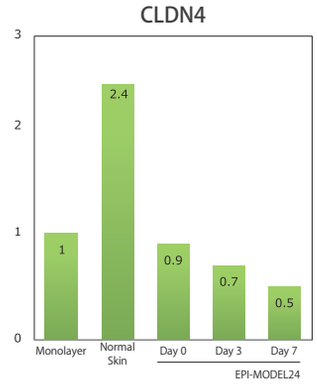
Fold of Control



Results demonstrate the formation of tight junctions during culture.

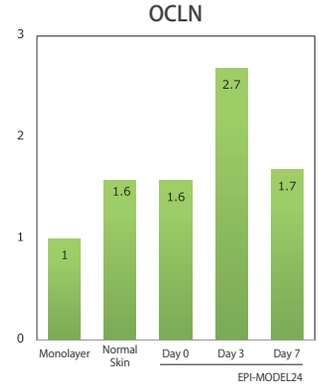


Results demonstrate the formation of tight junctions during culture.

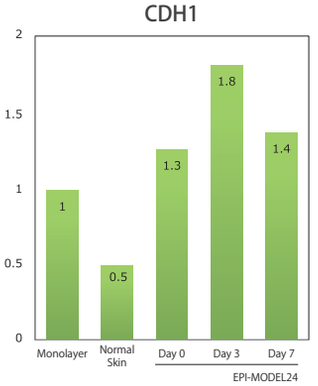


Results demonstrate the formation of tight junctions during culture.

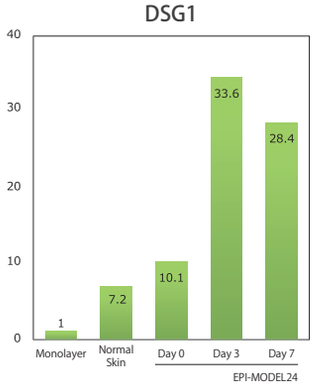
Fold of Control



Results demonstrate the formation of tight junctions during culture.

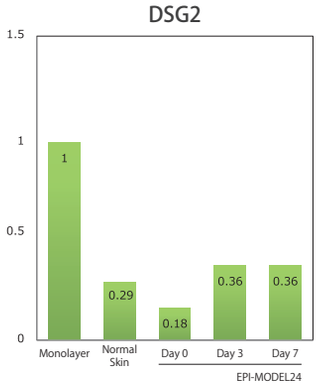


Results demonstrate the formation of adherens junctions during culture.

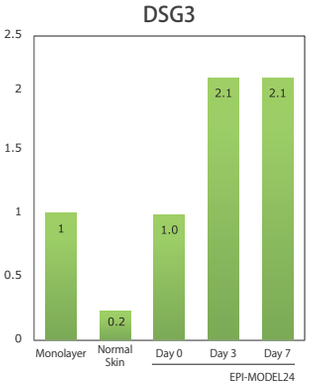


Results demonstrate the formation of desmosomes during culture.

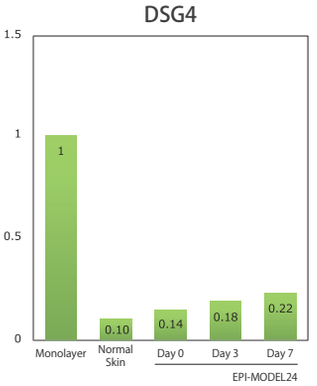
Fold of Control



Results demonstrate the formation of desmosomes during culture.

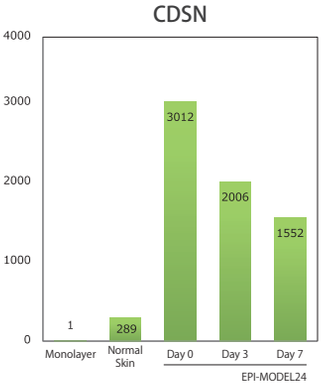


Results demonstrate the formation of desmosomes during culture.

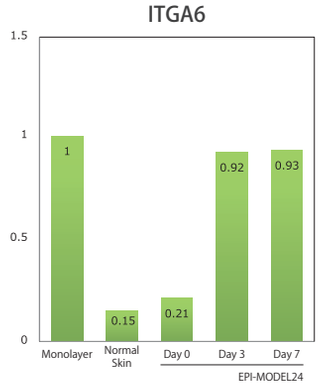


Results demonstrate the formation of desmosomes during culture.

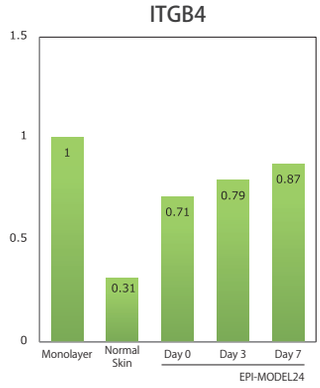
Fold of Control



Results demonstrate the formation of desmosomes during culture.

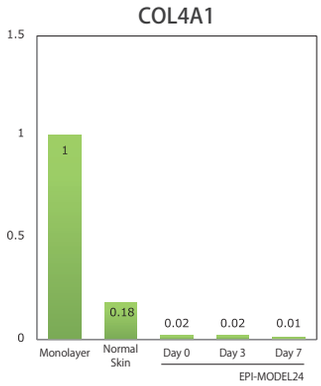


Results demonstrate the maintenance of the basal layer during culture.

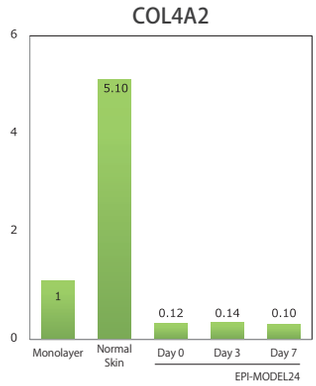


Results demonstrate the maintenance of the basal layer during culture.

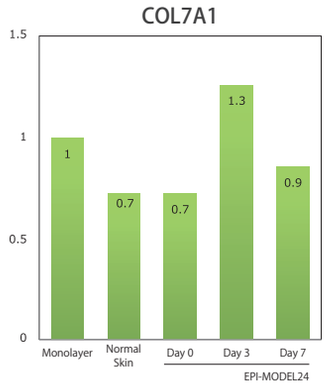
Fold of Control



Detection of COL4A1 expression during culture.

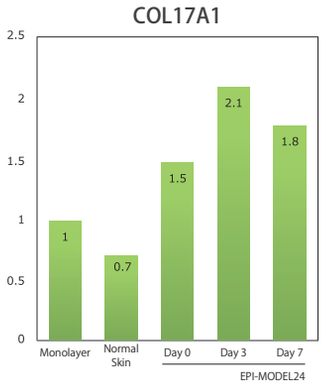


Detection of COL4A2 expression during culture.

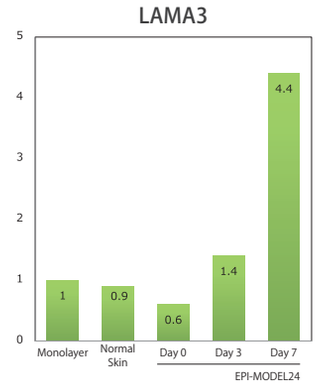


Detection of COL7A1 expression during culture.

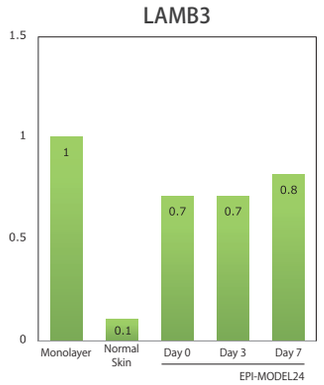
Fold of Control



Detection of COL17A1 expression during culture.

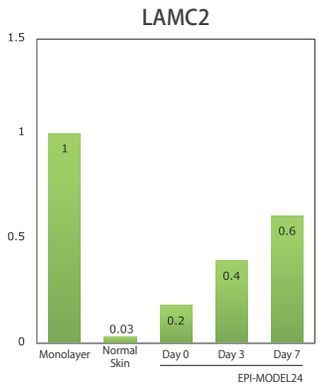


Detection of the Laminin-332 component LAMA3.

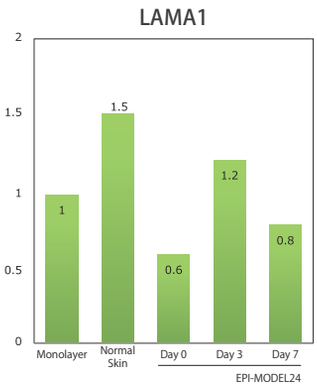


Detection of the Laminin-332 component LAMB3.

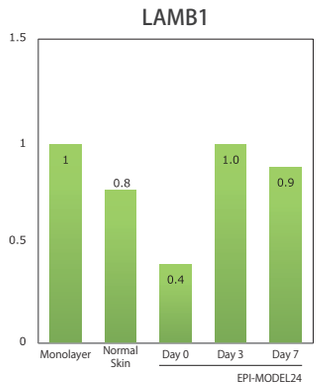
Fold of Control



Detection of the Laminin-332 component LAMC2.

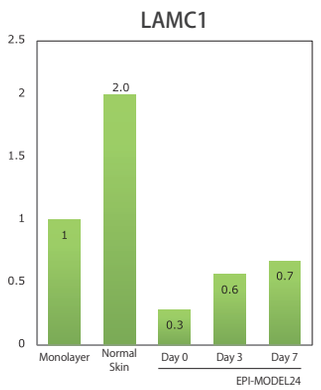


Detection of the Laminin-111 component LAMA1.



Detection of the Laminin-111 component LAMB1.

Fold of Control



Detection of the Laminin-111 component LAMC1.

Skin Irritation and corrosionTest

LabCyte EPI-MODEL 24 was accepted by the OECD to be used in the irritancy and corrosivity assessment of chemical substances under the test guideline 439 and 431.

Skin irritation test set
(Product No.: 401151)

The OECD Guidelines are internationally agreed testing methods used by government, industry and independent laboratories to identify and characterize potential hazards of chemicals.
OCED Test Guideline 439 and 431 describe in vitro procedures that may be used for the hazard identification of chemicals (substances and mixtures) using reconstructed human epidermis that closely mimics the biochemical and physiological properties of the outermost layer of the human skin.

Concordance with in vivo classification

- Skin irritation test

		in vivo classification		
		irritant	Non-irritant	Total
in vitro prediction	irritant	16	11	27
	Non-irritant	1	26	27
	Total	17	37	54
Sensitivity(%)		94.1		
Specificity(%)		70.3		
Accuracy(%)		77.8		

- Skin corrosion test

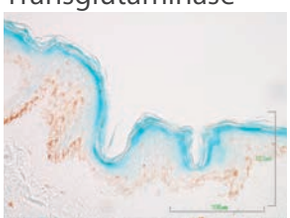
		in vivo classification			
		1A	1B/C	Non-corrosive	Total
in vitro prediction	1A	31	27	3	61
	1B/C	5	63	21	89
	Non-corrosive	0	0	87	87
	Total	36	90	111	237

Correct Classifications:	
1A correctly classified	86.1%
1B-and-/1C correctly classified	70.0%
NC correctly classified	78.4%
Overall Accuracy	76.4%

Immunohistochemistry of cultured EPI-MODEL 6D

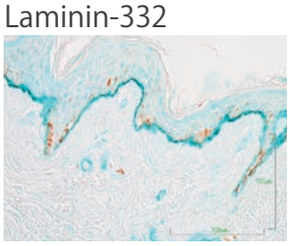
Human skin	EPI-MODEL24 6D	
	Day 0	Day 3

Transglutaminase



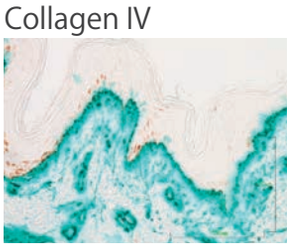
Expression detected from spinous to cornified layer.

Laminin-332



Expression detected in the basal and spinous layers
as culture progressed.

Collagen IV

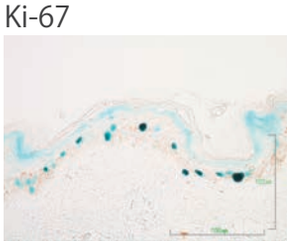


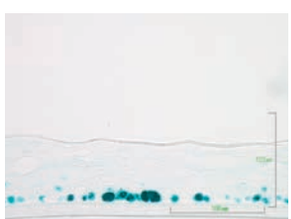
Expression detected in the basal layer.

Collagen VII

Expression detected in the basal layer.

Ki-67





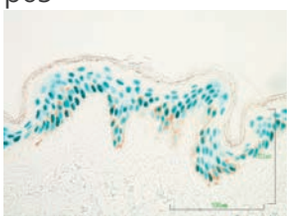
Ki67 is a widely used proliferation marker.
Expression detected in the cells of the basal layer,
as it is observed in human skin.

Human skin

EPI-MODEL24 6D

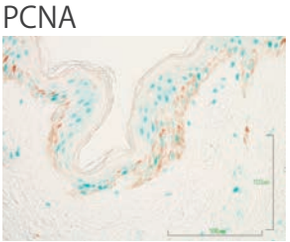
Day 0 Day 3

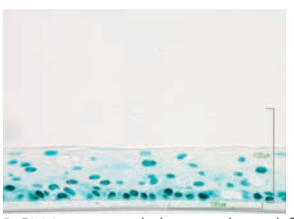
p63

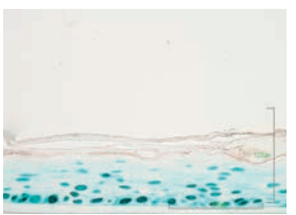


p63 is often referred to as a marker for epidermal stem cells.
Expression detected in the basal and spinous layers,
as it is described in human skin.

PCNA





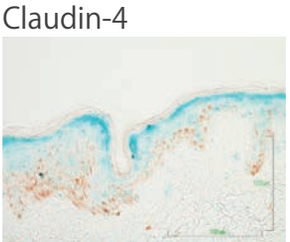


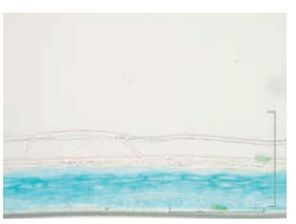
PCNA is a widely used proliferation marker.
Expression detected in the cells of the basal layer,
as it is described in human skin.

Claudin-1

Claudins are tight junction markers.
Expression detected from basal to granular layer,
similar to human skin.

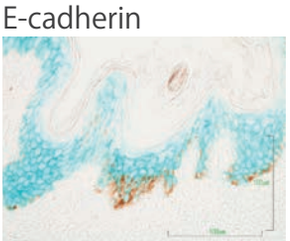
Claudin-4

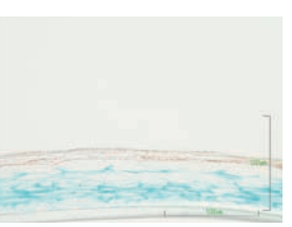




Expression detected from basal to granular layer,
similar to human skin.

E-cadherin

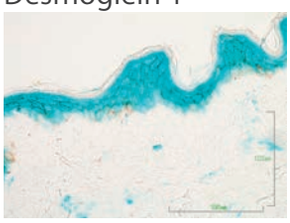




E-cadherin is an adherens junction marker.
Expression detected from basal to granular layer,
similar to human skin.

Human skin	EPI-MODEL24 6D	
	Day 0	Day 3

Desmoglein 1

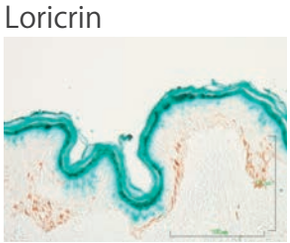


Desmoglein is a desmosome marker.
Expression detected between the basal and granular layer, similar to human skin.

Filaggrin

Expression detected from the granular layer.

Loricrin

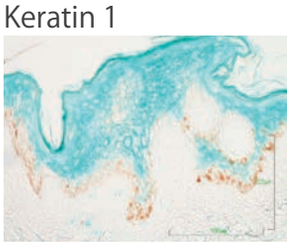


Expression detected in the granular layer, similar to human skin.

Involucrin

Involucrin is an epidermal differrentiation marker.
Unlike in human skin, expression was detected in all layers of the epidermal tissue.

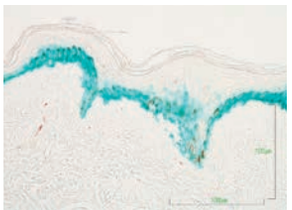
Keratin 1



Keratin 1 is an epidermal differentation marker.
Expression detected from spinous to cornified layer, similar to human skin.

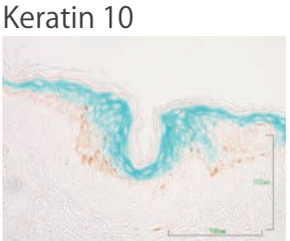
Human skin	EPI-MODEL24 6D	
	Day 0	Day 3

Keratin 5



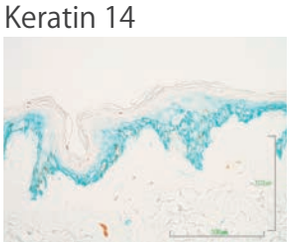
Keratin 5 is a basal layer marker.
Expression detected in the basal layer, similar to human skin.

Keratin 10



Keratin 10 is an epidermal differentation marker.
Expression detected from spinous to cornified layer,
similar to human skin.

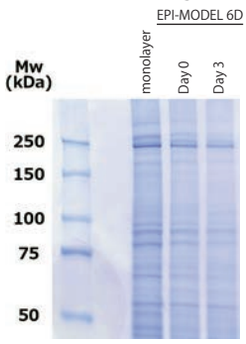
Keratin 14



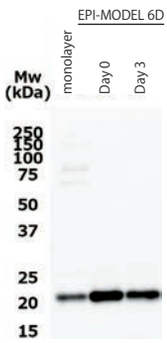
Keratin 14 is a basal and spinous layer marker.
Expression detected in the basal and spinous layers,
similar to human skin.

Western blotting analysis of cultured EPI-MODEL 6D

CBB staining

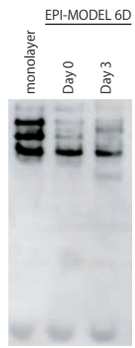


Claudin-1

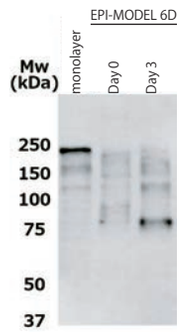


Results indicate the presence of tight junctions

Native-PAGE

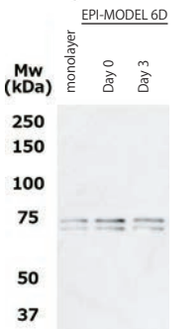


Collagen IV



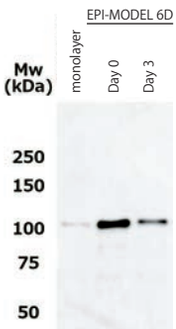
Results indicate the production of collagen IV

Collagen XVII



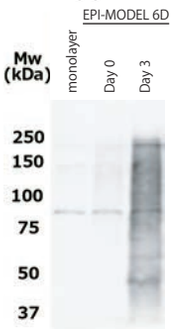
Results indicate the production of collagen XVII

E-Cadherin



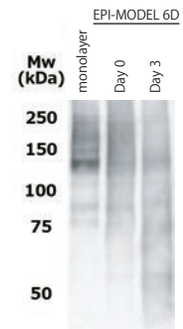
Results indicate the presence of adherens junction

Filaggrin



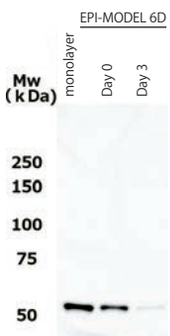
Results demonstrate the maturation of the cornified layer.

Involucrin



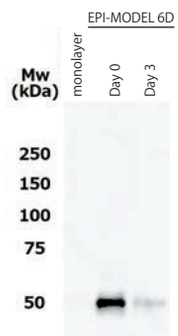
Results demonstrate the maturation of the cornified layer.

Keratin 5



Results demonstrate the maintenance of the basal layer during culture.

Keratin 10

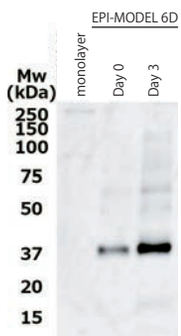


Results demonstrate the differentiation process induced by the 3D culture of epidermal cells.

Keratin 14

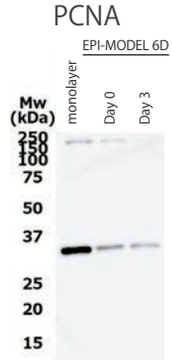
Results demonstrate the maintenance of the basal layer during culture.

Loricrin



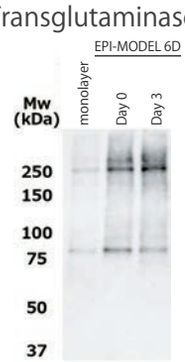
Results demonstrate the differentiation process induced by the 3D culture of epidermal cells.

PCNA



Results demonstrate the decrease of proliferating cells during culture.

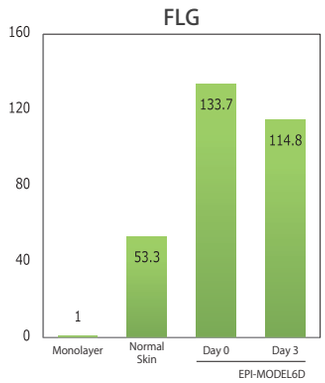
Transglutaminase



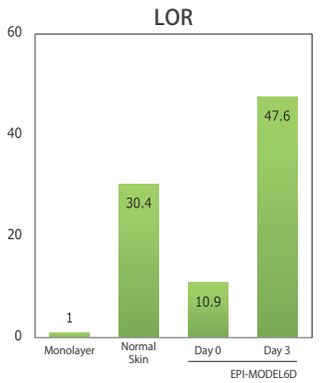
Results demonstrate the maturation of the cornified layer.

Gene expression profile of EPI-MODEL6D during culture

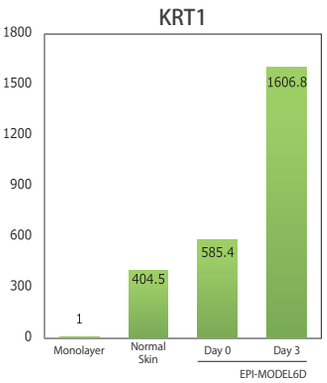
Fold of Control



Results demonstrate the maturation of the cornified layer.

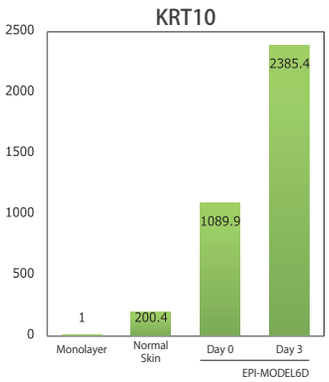


Results demonstrate the maturation of the cornified layer.

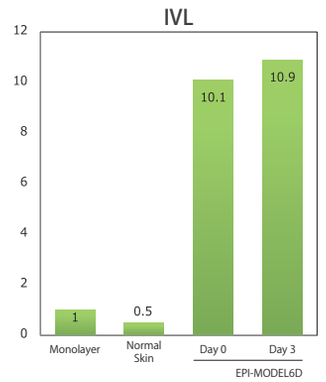


Results demonstrate the maturation of the cornified layer.

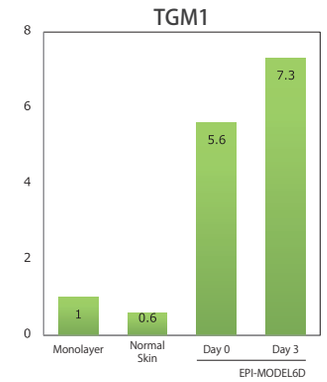
Fold of Control



Results demonstrate the maturation of the cornified layer.

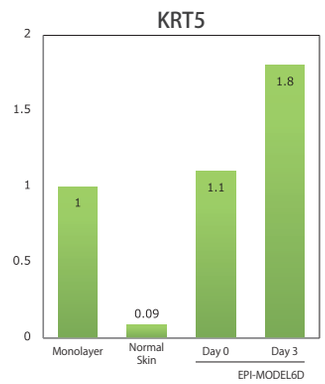


Results demonstrate the maturation of the cornified layer.

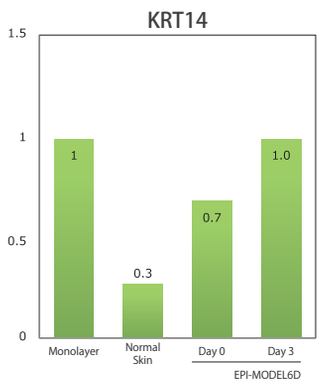


Results demonstrate the maturation of the cornified layer.

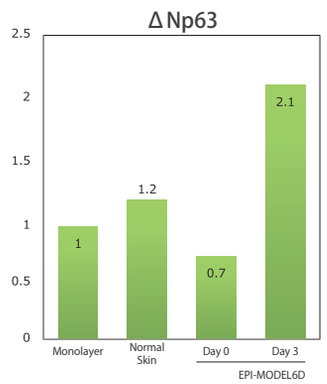
Fold of Control



Results demonstrate the maintenance of the basal layer during culture.

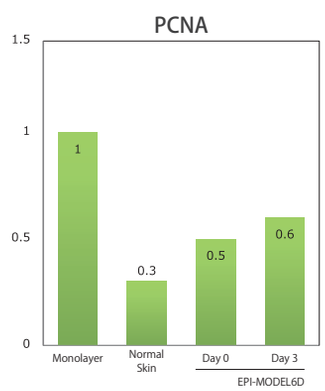


Results demonstrate the maintenance of the basal layer during culture.

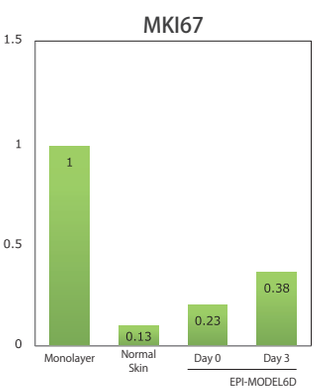


Results demonstrate the maintenance of epidermal stem cells during culture.

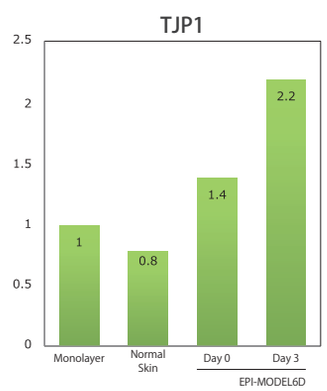
Fold of Control



Results demonstrate the increase of proliferating cells during culture.

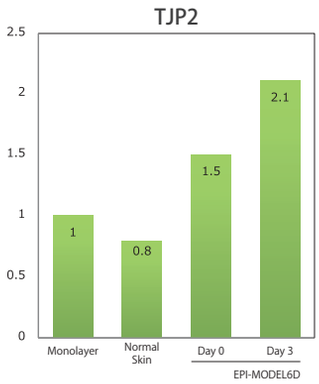


Results demonstrate the increase of proliferating cells during culture.

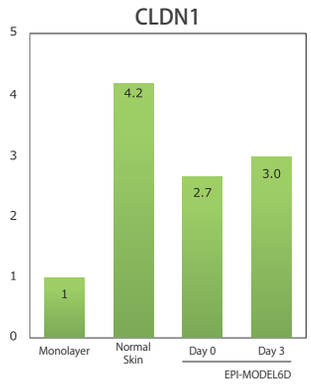


Results demonstrate the formation of tight junctions during culture.

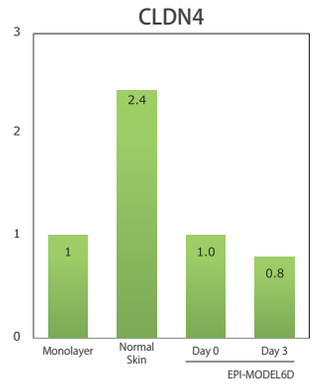
Fold of Control



Results demonstrate the formation of tight junctions during culture.

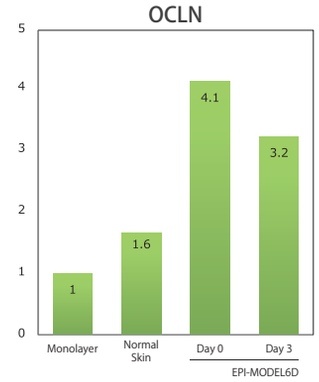


Results demonstrate the formation of tight junctions during culture.

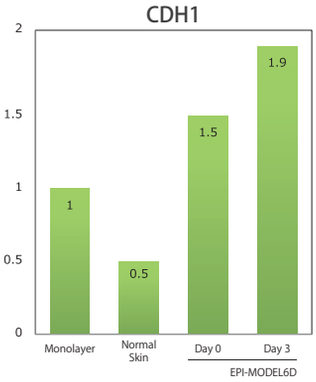


Results demonstrate the formation of tight junctions during culture.

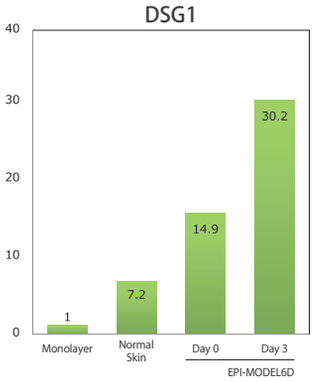
Fold of Control



Results demonstrate the formation of tight junctions during culture.

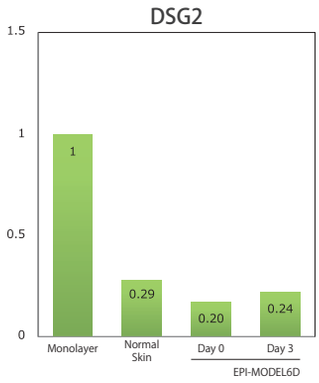


Results demonstrate the formation of adherens junctions during culture.

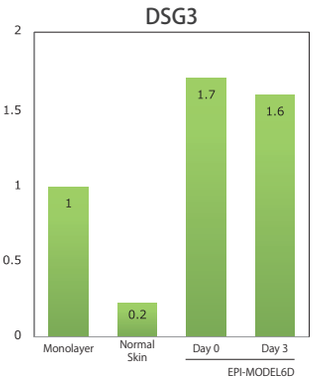


Results demonstrate the formation of desmosomes during culture.

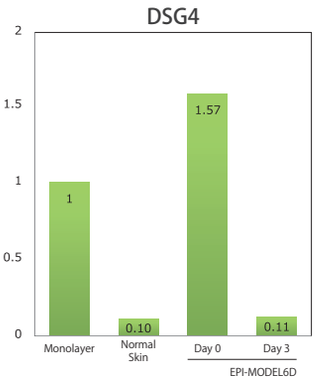
Fold of Control



Results demonstrate the formation of desmosomes during culture.

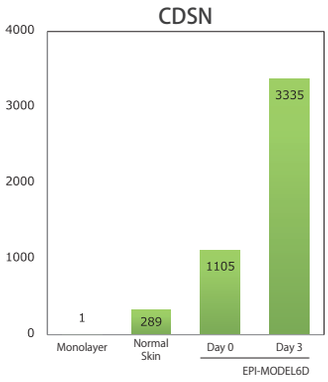


Results demonstrate the formation of desmosomes during culture.

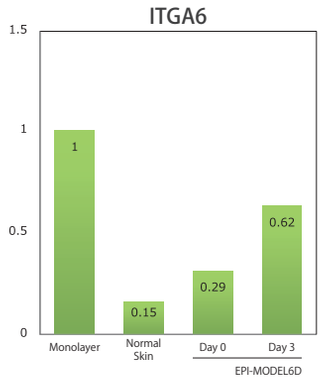


Results demonstrate the formation of desmosomes during culture.

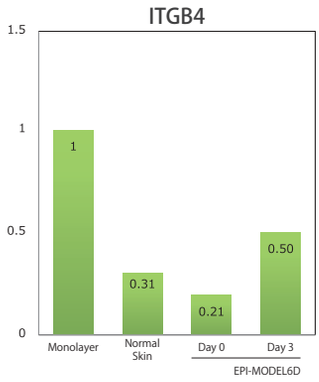
Fold of Control



Results demonstrate the formation of desmosomes during culture.



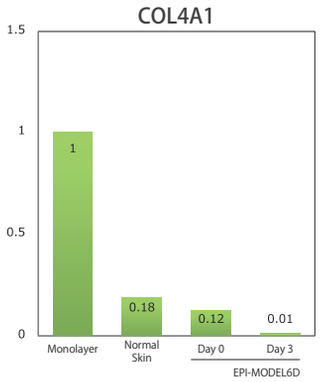
Results demonstrate the maintenance of the basal layer during culture.



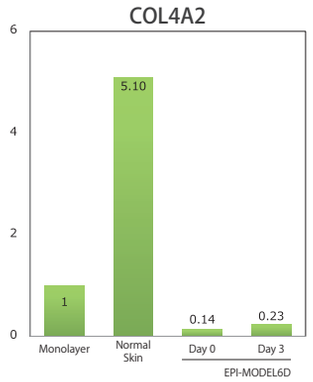
Results demonstrate the maintenance of the basal layer during culture.

EPI-MODEL 6D

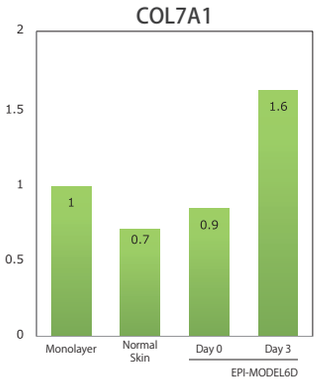
Fold of Control



Detection of collagen 4A1 expression during culture.

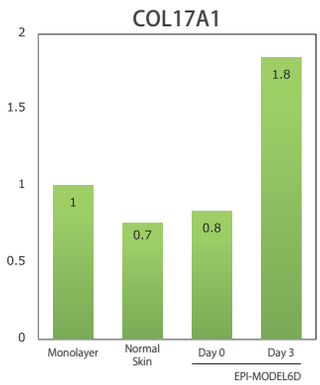


Detection of collagen 4A2 expression during culture.

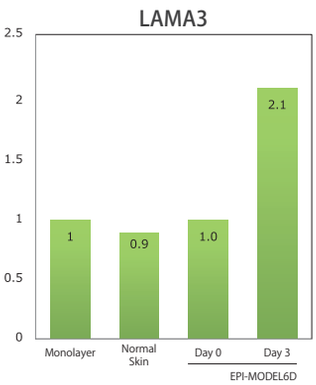


Detection of collagen 7A1 expression during culture.

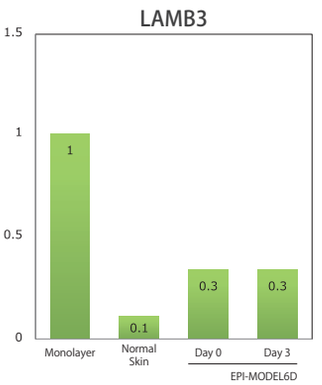
Fold of Control



Detection of collagen 17A1 expression during culture.

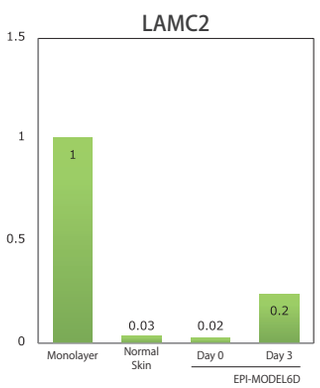


Detection of the Laminin-332 component LAMA3.

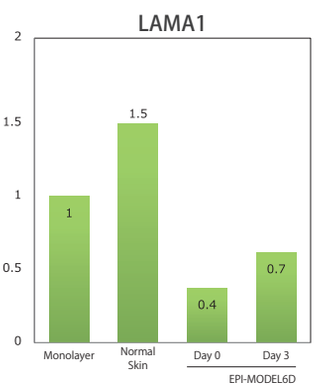


Detection of the Laminin-332 component LAMB3.

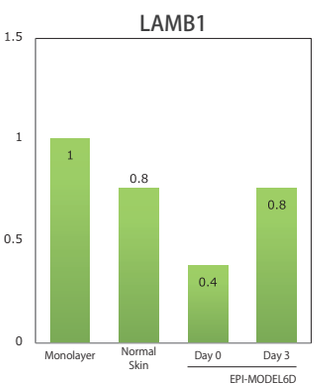
Fold of Control



Detection of the Laminin-332 component LAMC2.

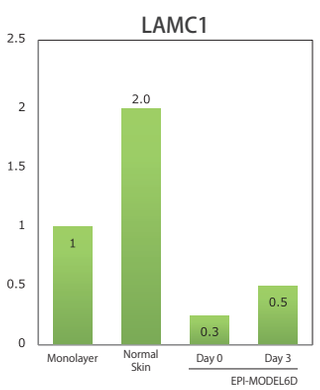


Detection of the Laminin-111 component LAMA1.



Detection of the Laminin-111 component LAMB1.

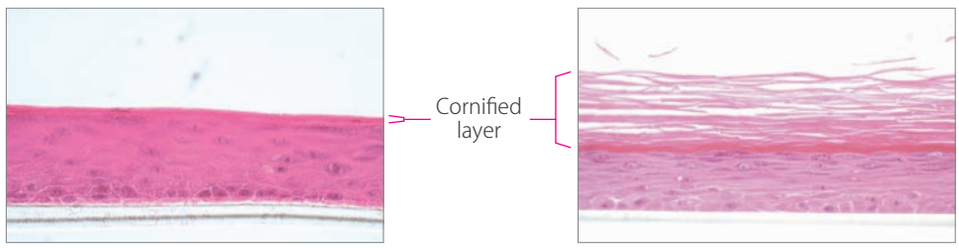
Fold of Control



Detection of the Laminin-111 component LAMC1.

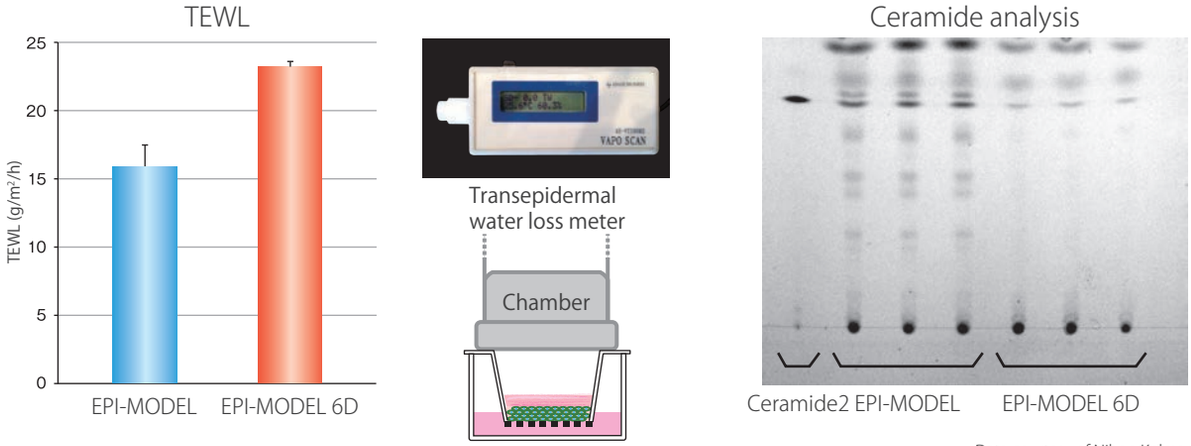
Test of mild irritants

Histology



LabCyte EPI-MODEL 6D　　LabCyte EPI-MODEL

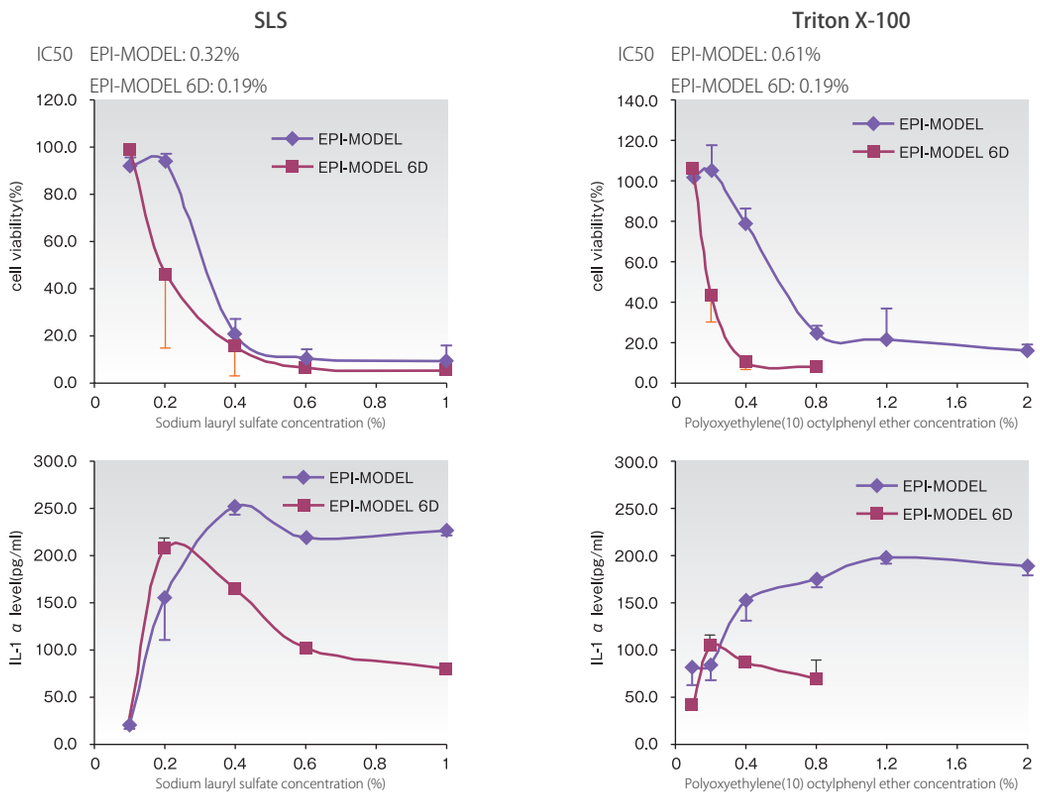
Barrier function assessment



Data courtesy of Nihon Kolmar Co., Ltd.
(J Soc Cosmet Chem Jpn. 2013, Vol 47, No. 1)

Test of detergents

Test substances: Sodium lauryl sulfate (SLS), Polyoxyethylene(10) octylphenyl ether (Triton X-100)
Protocol: 15 min exposure, 42 h post-exposure incubation



Data courtesy of Nihon Kolmar Co., Ltd.
(J Soc Cosmet Chem Jpn. 2013, Vol 47, No. 1)

Test of mild irritants

Evaluation of cosmetic ingredients

Test substances

Test substance	Skin irritation index of human patch test
Lotion 1	10
Lotion 2	0
Lotion 3	1.3
Essence 1	3.8
Essence 2	1.3
Essence 3	0
Cream 1 (w/o)	0
Cream 2 (o/w)	0
Cream 3 (w/o)	2.5

Lotion

Class	Ingredients	Conc. (%)
A	Butylene glycol	9
	PEG-8	3
	PEG-60 hydrogenated castor oil	0.5
	※(Methylparaben)	(0.2)
	(Phenoxyethanol)	
	(Ethylhexylglycerin)	
	Citric acid	0.01
	Sodium citrate	0.09
	Water	remaining vol.
		100

※ Only when phenoxyethanol is also included

Cream (o/w)

Class	Ingredients	Conc. (%)
A	Polyglyceryl-2-isostearate	2
	Dextrin palmitate	1
	Petrolatum	2
	Mineral oil	40
	Beeswax	2
	(Ethylhexylglycerin)	
B	Glycerin	10
	Monosodium glutamate	1
	※(Methylparaben)	(0.2)
	(Phenoxyethanol)	
	(Pentylene glycol)	
	Water	remaning vol.
		100

Cream (w/o)

Class	Ingredients	Conc. (%)
A	Polyglyceryl-10 myristate	2.1
	Hydrogenated lecithin	0.6
	Behenyl alcohol	1.6
	Stearic acid	0.5
	Cetyl ethylhexanoate	6.6
	Mineral oil	2
	Dimethicone	0.2
	Glycerin	5
	※(Methylparaben)	(0.2)
	(Phenoxyethanol)	
	(Pentylene glycol)	
	(Ethylhexylglycerin)	
B	Water	remaining vol.
C	Carbomer	0.1
	Water	10
D	Sodium hydroxide	0.04
	Water	4.96
		100

Modification of exposure time

Test substances: cosmetic ingredients evaluated by the human patch test.
Protocol: 24 h exposure

Test substances	15 min exposure 42 h post incubation				24 h exposure			
	EPI-MODEL		EPI-MODEL 6D		EPI-MODEL		EPI-MODEL 6D	
	Cell viability (%)		Cell viability (%)		Cell viability (%)		Cell viability (%)	
	Mean±SD	Result	Mean±SD	Result	Mean±SD	Result	Mean±SD	Result
Lotion 1	99.8±9.8	NI	85.2±15.1	NI	12.5±2.0	I	5.8±0.5	I
Lotion 2	101.9±9.7	NI	89.8±11.3	NI	107.4±3.6	NI	34.2±24.7	I
Lotion 3	98.5±4.6	NI	88.5±15.7	NI	125.5±13.4	NI	78.2±11.6	NI
Essence 1	103.2±6.9	NI	105.5±7.1	NI	40.0±34.0	I	5.5±3.8	I
Essence 2	105.0±8.7	NI	93.9±27.5	NI	36.3±16.3	I	17.4±8.5	I
Essence 3	103.3±1.8	NI	96.0±8.3	NI	107.6±12.0	NI	104.6±9.1	NI
Cream 1 (w/o)	95.1±16.6	NI	100.9±0.7	NI	108.9±13.0	NI	86.3±29.0	NI
Cream 2 (o/w)	102.7±7.4	NI	98.6±7.4	NI	66.4±33.3	NI	17.3±2.9	I
Cream 3 (w/o)	106.5±6.5	NI	99.1±0.5	NI	121.4±7.4	NI	63.1±15.4	NI

Results interpretation
Cell Viability ≦ 50%　irritant (I)
Cell Viability ＞ 50%　non irritant (NI)

Data courtesy of Nihon Kolmar Co., Ltd.
(J Soc Cosmet Chem Jpn. 2013, Vol 47, No. 1)

Evaluation of antiseptic ingredients

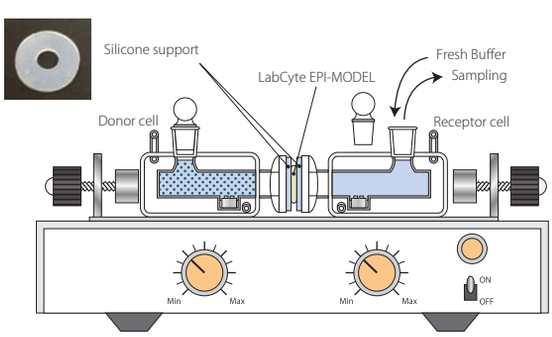
Test substances: cosmetic ingredients containing antiseptics.
Human patch test index unkown.
Protocol: 24 h exposure

Antiseptic ingredient	Concentration and formulation	24 h exposure			
		EPI-MODEL		EPI-MODEL 6D	
		Cell viability (%)		Cell viability (%)	
		Mean±SD	Result	Mean±SD	Result
Phenoxyethanol (0.2% Methylparaben)	0.5% Lotion	113.6±27.8	NI	74.7±35.6	NI
	0.1% Lotion	122.5±3.7	NI	119.1±16.3	NI
	0.5% Cream (o/w)	78.6±7.7	NI	19.6±5.6	I
	0.1% Cream (o/w)	115.0±10.0	NI	52.0±18.3	NI
	0.5% Cream (w/o)	74.3±24.7	NI	64.8±23.4	NI
	0.1% Cream (w/o)	107.7±13.3	NI	107.5±11.8	NI
Pentylene glycol	5% Lotion	111.4±16.1	NI	33.9±6.9	I
	2% Lotion	108.2±13.9	NI	107.0±3.5	NI
	5% cream (o/w)	113.8±14.6	NI	49.5±4.0	I
	2% cream (o/w)	98.7±10.1	NI	113.9±10.9	NI
	5% cream (w/o)	104.1±16.4	NI	112.5±4.6	NI
	2% cream (w/o)	97.0±11.7	NI	107.0±4.2	NI
Ethylhexylglycerin	0.1% Lotion	102.5±3.7	NI	55.1±4.6	NI
	0.02% Lotion	102.5±8.6	NI	109.1±6.2	NI
	0.1% Cream (o/w)	107.8±4.7	NI	107.2±10.3	NI
	0.02% Cream (o/w)	101.0±5.3	NI	108.6±5.1	NI
	0.1% Cream (w/o)	92.2±2.8	NI	94.4±2.3	NI
	0.02% Cream (w/o)	94.2±6.2	NI	92.6±4.7	NI

Permeability test

Skin permeability test using LabCyte EPI-MODEL

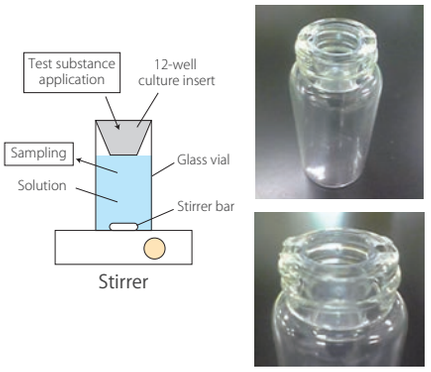
Skin permeability tests can be performed by sandwiching the LabCyte EPI-MODEL between silicone elastomer adapters, and then mounting the adapters between side-by-side diffusion cells.

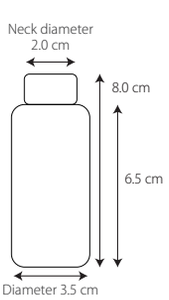


Data courtesy of Dr. T. Hikima (Kyushu Inst. Tech.)
Biol. Pharm. Bull. 35(3) 362—368 (2012)

Percutaneous absorption test

Percutaneous absorption test cab be performed by simply attaching the culture insert to a glass vial.



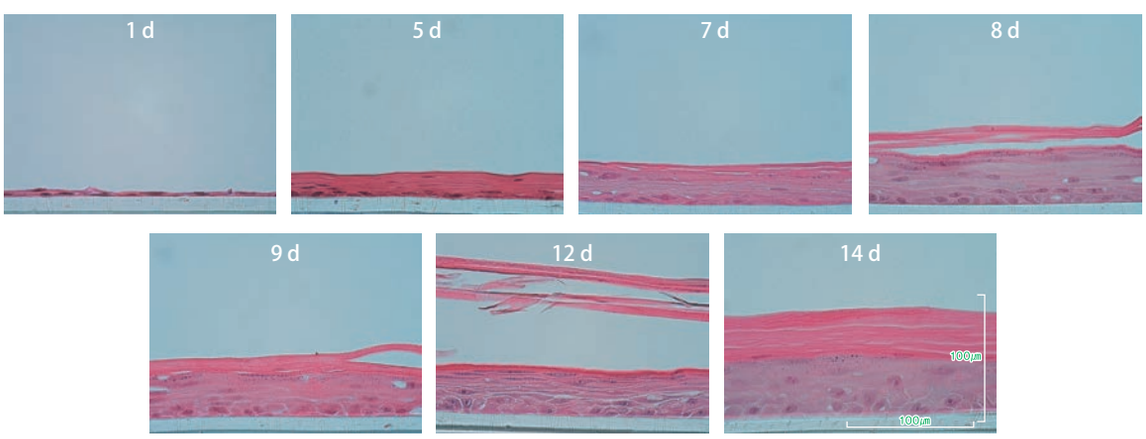


Dimensions compatible with EPI-MODEL12

Data courtesy of Dr. S. Fukushima (Kobe Gakuin Univ.)

Prepare your own epidermal model by simply seeding and culturing keratinocytes in cell culture inserts

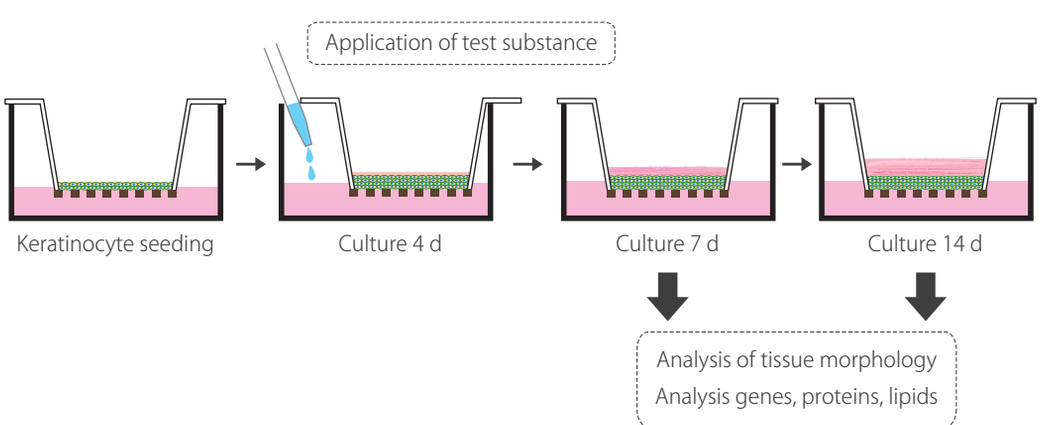
EPI-KIT
(Product No.: 401810)



Proliferation and differentiation rates vary according to culture conditions.
The data above should be used as reference only.
Assessing sample histology during culture is highly recommended.

Application example

■ Apply test substances and perform model analysis at a time of your convenience.



Gene expression knockdown in epidermal models prepared with LabCyte EPI-KIT

■ Introduction

The experiment presented here (fillagrin knockdown) was designed based on information available in the literature. Please use the protocol below as a reference for gene expression knockdown in cultured epidermal models.

■Reference

Journal of Investigative Dermatology vol.130 2286-2294 (2010)
Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model.
Mildner M et., al.

■ Material

siRNA-1 (filaggrin targetting sequence siRNA-1)
sense：5-GAGGUGGUCUGGGUCUGCUUCCAGA-3
antisense：5-UGGAAGCAGACCCAGACCACCUCUC-3

siRNA-2（filaggrin targetting sequence siRNA-2）
sense：5-ACAGAAAGCACAGUCAUCAUGAUAA-3
antisense：5-AUCAUGAUGACUGUGCUUUCUGUGC-3

siRNA-3（filaggrin targetting sequence siRNA-3）
sense：5-GAGGUUGUCUGGGUCUGCUUCCAGA-3
antisense：5-UGGAAGCAGACCCAGACAACCUCUC-3

ctrl-siRNA（siRNA control sequence）
sense：5-GAGUGGGUCUGGGUCUUCCCGUAGA-3
antisense：5-UACGGGAAGACCCAGACCCACUCUC-3

Opti-MEM®
Life Technologies, Code 31985-070, 500ml

Lipofectamine®2000 Transfection Reagent
Life Technologies, Code11668-027, 0.75ml

■ Protocol

The measurements below are for the preparation of one 24-well plate using one siRNA sequence. Measurements should be adjusted for fewer wells.

1. Preparation of siRNA-Lipofectamine® complex

Prepare the complex in 15 ml tubes, under sterile conditions, using the measurements below.

siRNA mixture
5 ml OPTI-MEM®
50 µl Lipofectamine® 2000
26 µl 100 µM siRNA

Prepare your Mock transfection sample without siRNA at this point, if necessary.
Leave the siRNA-Lipofectamine® complex resting at room temperature for 30 min.

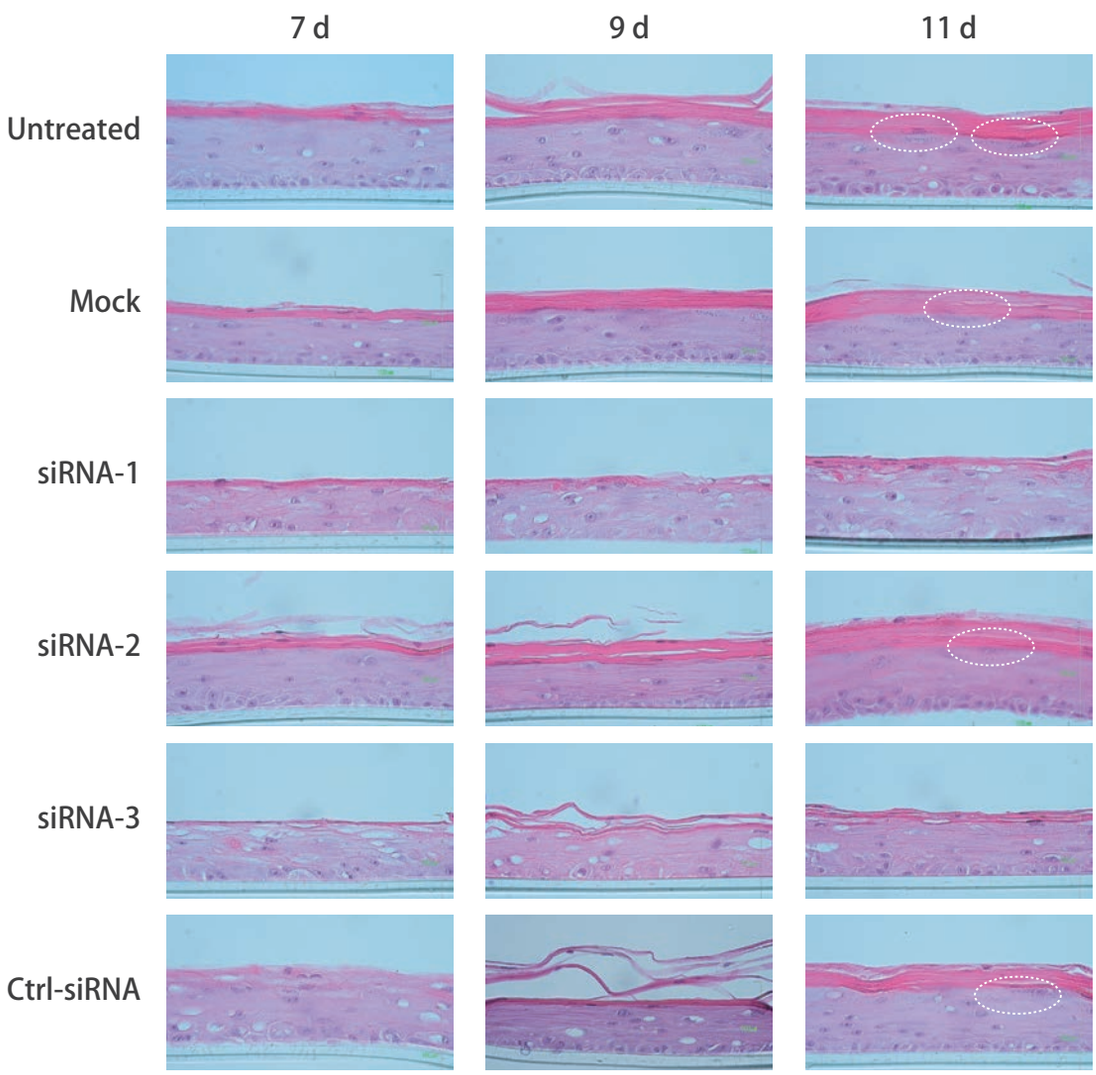
2. Transfection

1. Thaw keratinocytes according to the EPI-KIT instruction manual.
2. Add 7 ml of assay medium to siRNA - Lipofectamine® complexes and mix well.
3. Add thawn keratinocytes to siRNA-containing mixture prepared in step 2.
4. Gently mix the solution prepared in step 3, and add 500 µl to each cell culture insert.

■ Results

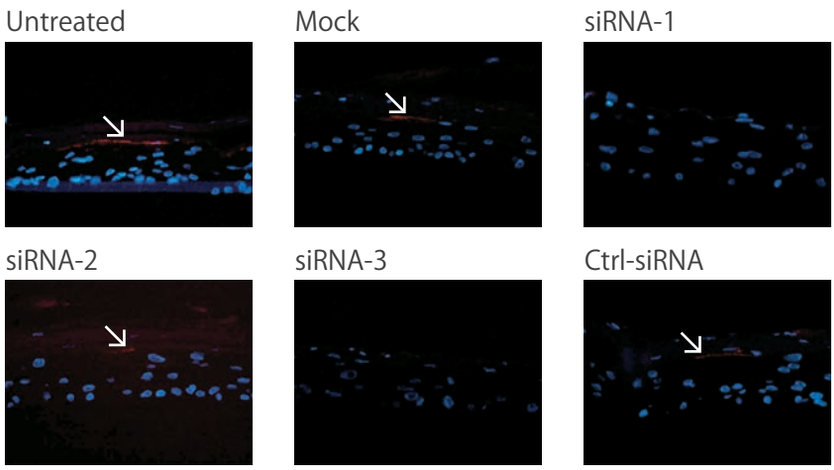
1. Epidermal model histology

Media was removed from the cell culture inserts, and keratinocyte culture was done according to the EPI-KIT instruction manual.



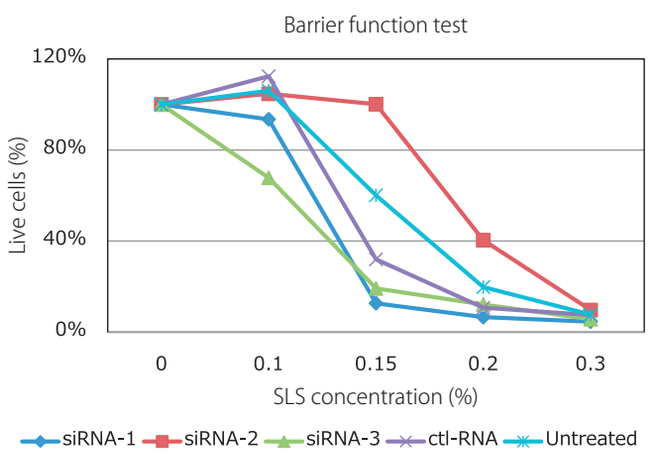
Cultured models were fixed in 4% Formalin Neutral Buffer Solution, at 7d, 9d, and 11d.
Untreated: Models prepared according to EPI-KIT standard protocols.
Mock: Models prepared with the addition of Lipofectamine®, without siRNA, before cell seeding.
siRNA-1, siRNA-2, siRNA-3, ctrl-RNA: Models prepared with correspondent siRNA-Lipofectamine® complex.
Dotted circles showing cells containing keratohyalin granules.
Cells containing keratohyalin granules not found in models transfected with siRNA-1 and siRNA-3.

2. Immunohistochemistry



Immunohistochemistry was performed on epidermal models fixed at 11 d.
Filaggrin staining (red) showed by white arrows.
Primary antibody: Anti-filaggrin antibody (Neuromics MO20041).
Secondary antibody: Alexa FluorR 546 Goat Anti-Mouse IgG (H+L) (Life Technologies A-11030).
Nuclear staining (blue): Hoechst 33258.
Filaggrin was not detected in models transfected with siRNA-1 and siRNA-3.

3. Epidermal barrier function assessment



Barrier function test was performed using models cultured for 13 d.
25 µl of SLS-PBS solution (SLS concentration: 0%, 0.1%, 0.15%, 0.2%, 0.3%) was added to the models (n = 3) that were then incubated in a CO_2 incubator for 18 h at 37° C.
After 18 h, models were rinsed with DPBS (add 0.5 ml of DPBS to the culture insert and discard - repeat 3 times). Rinsed models were processed for MTT assay. Data were normalized by results from 0% SLS treatment (0% SLS cell viability = 100%).
Average of 3 readings (n = 3) plotted in the graph above.
Model transfected with siRNA-2 showed higher barrier function compared to untreated model.

Note:
The results of this experiment differ from those of the reference article (siRNA-1, siRNA-2, siRNA-3 show the same results).

CORNEA-MODEL

LabCyte CORNEA-MODEL is a 3-D human cultured corneal epithelial tissue produced from normal human corneal epithelial cells. LabCyte CORNEA-MODEL was developed by applying cell culture techniques to differentiate and stratify corneal epithelial cells to form a tissue structure similar to that of the normal human cornea. LabCyte CORNEA-MODEL can be used in the hazard identification of irritant chemicals by the eye irritation test. Moreover, CORNEA-MODEL can also be used to evaluate biological processes, such as the production of mucin and the expression of adhesion molecules, in response to different test substances.

Model features

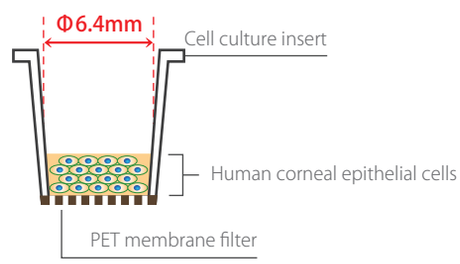
3-D culture of human corneal epithelial cells

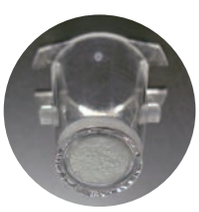
Can be used for eye irritation tests.
Enables the analysis of proteins specifically expressed in the corneal epithelium.

Low lot-to-lot variability

Developed and manufactured at J-TEC using cell culture techniques fostered in house.

Model structure



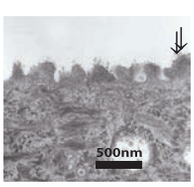


Model appearance

Reproducing the corneal epithelial structure

HE staining

Cell differentiation and stratification.



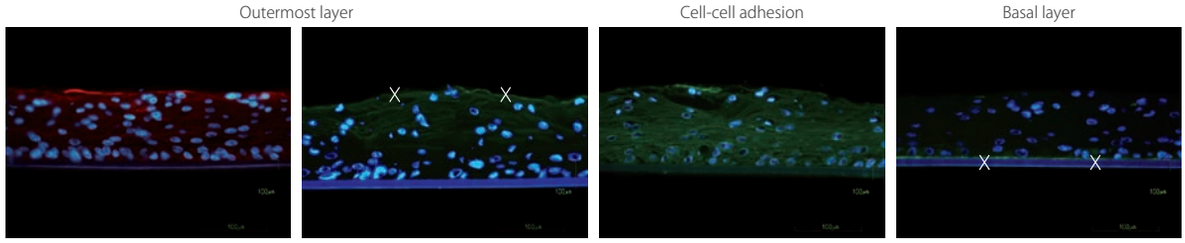
Transmission electron microscopy

Observation of microvilli and glycocalyx (arrows) on the outermost layer of cells.

Immunohistochemistry

Detection of proteins expressed at different cells layers of the corneal epithelial.

Outermost layer | Cell-cell adhesion | Basal layer



Mucin-1 | Mucin-16 | Desmoglein-3 | Laminin V

OECD Test Guideline 492 - Eye Irritation Test

LabCyte CORNEA-MODEL was accepted by the OECD to be used in the eye irritancy assessment of chemical substances under the test guideline 492.
The OECD test guidelines are internationally accepted testing methods used by government, industry and independent laboratories to identify and characterize potential hazards of chemicals. OCED test guideline 492 describes an in vitro procedure that may be used for the hazard identification of eye irritant chemicals using reconstructed human cornea-like epithelium that closely mimics the biochemical and physiological properties of the outermost layer of the human corneum epithelium.

Eye irritation test set
(Product No.: 401351)

Test procedure

① Pre-incubation
② Application of test substance,rinsing, and post-exposure incubation
(conditions differ according to the physical state of the tested chemical)
③ Cell viability measurement (WST-8 assay)
④ Determine whether results meet acceptance criteria
❶ Cell viability: 0.5 ≦ mean OD (A450/650) measured value for negative control ≦ 1.6
❷ Positive control: mean tissue viability for positive control ≦ 40%
❸ SD: SD (negative control and positive control) of tissue viability of three indentical replicates ≦ 18

Classification criteria

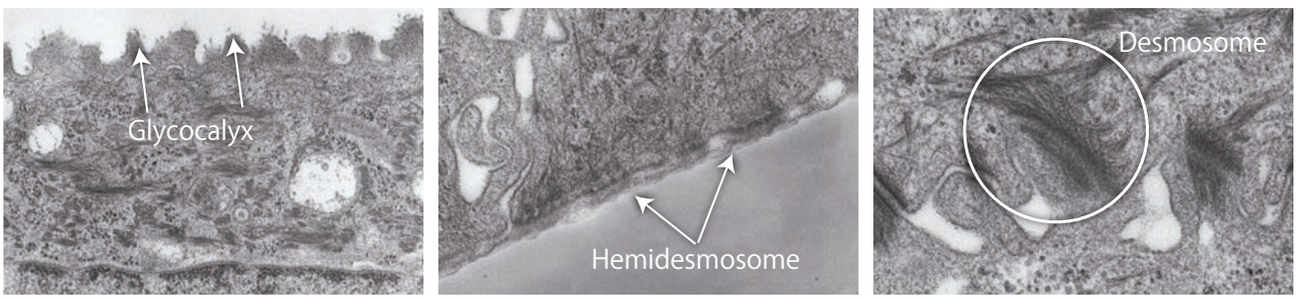
Tissue viability ≦ 40%	Category 1 or 2 (irritant)
Tissue viability > 40%	Non Category (non-irritant)

Concordance with in vivo classification

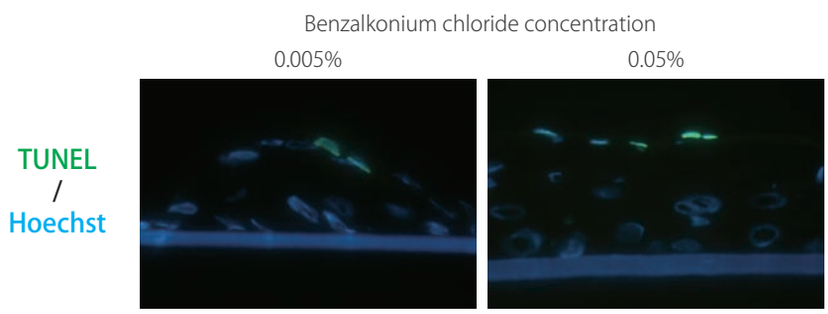
		in vivo classification		
		irritant	Non-irritant	Total
in vitro prediction	irritant	76	17	93
	Non-irritant	0	46	46
	Total	76	63	139
Sensitivity(%)		100		
Specificity(%)		73.0		
Accuracy(%)		87.8		

LabCyte CORNEA-MODEL can be used to study histological features of the corneal epithelium.

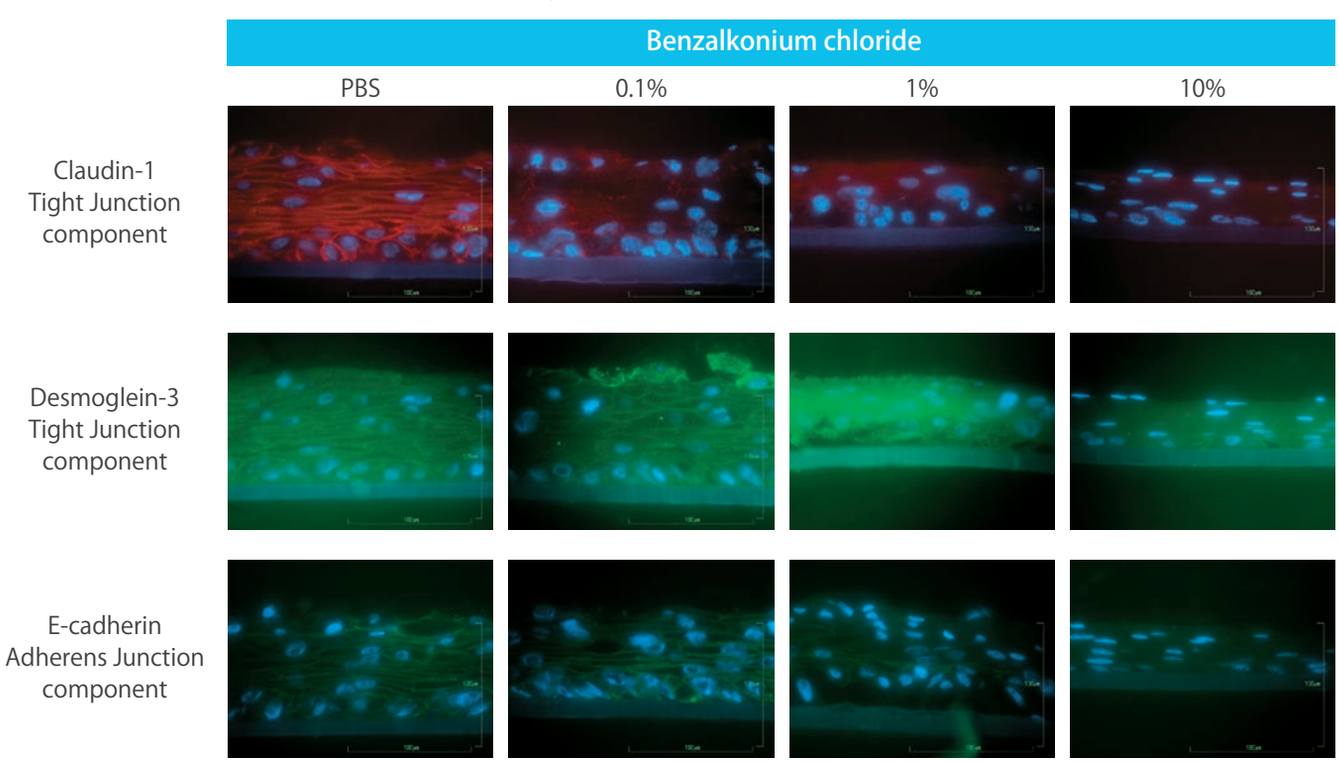
LabCyte CORNEA-MODEL reproduces microstructural features of the corneal epithelium.



Apoptosis induction by benzalkonium chloride application assessed by TUNEL staining



Disruption of cell-cell adhesion promoted by benzalkonium chloride



Disruption of cell-cell adhesion by benzalkonium chloride is concentration-dependent

March 2021.

Product features may change without prior notice.

Through the development of tissue-engineered medical products, J-TEC will contribute to the development of techniques that can be used as alternatives to animal testing.

J-TEC

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