SkinEthic™ RHPE | Data Sheet

Reconstructed Human Pigmented Epidermis

Features

- Co-Culture of Human Keratinocytes and Melanocytes
- Available in Phototypes II, IV, and VI
- Chemically Defined, Serum-Free Medium
- Air-Liquid Interface Culture System
- Human Skin-Like 3D Structure
- Highly Reproducible
- Progressive, Macroscopic Pigmentation
- Easily Handled Cell Culture Inserts (0.5 cm^2)
- Quantifiable, Objective Endpoints (Melanin Content, Histology)
- Cost-Effective Alternative to Clinical Testing
- Suitable for Depigmentation and Pigmentation Assays
- Validated with Fontana Masson and H&E Staining

The SkinEthic™ RHPE Model

The SkinEthic™ Reconstructed Human Pigmented Epidermis (RHPE) consists of normal human keratinocytes co-cultured with melanocytes at the air-liquid interface in a chemically defined, serum-free medium on inert polycarbonate filters. The keratinocyte-to-melanocyte seeding ratio is 10:1, and tissues are cultured for 10 days to form a fully differentiated, multilayered 3D human epidermal structure.

The melanocytes within the co-cultures undergo spontaneous melanogenesis, producing tissues of varying pigmentation levels without the need for artificial stimulators such as TPA or IBMX. The different tanning degrees of these constructs correspond macroscopically to three distinct human skin phototypes: II (light), IV (intermediate), and VI (dark). Cultures containing melanocytes from darker phototype donors show increased pigmentation compared to those from lighter phototypes.

The RHPE model provides a useful in vitro means to evaluate cosmetic and pharmaceutical agents designed to modulate skin pigmentation. The organotypic cultures allow for topical application of melanogenesis inhibitors or stimulators. Analytical methods include evaluation of melanocyte dendricity, pigment granule transfer to adjacent keratinocytes, bulk darkening of tissue, and total melanin content quantification. The model offers a cost-effective and ethical alternative to clinical and whole animal testing for assessing skin pigmentation modulation.

Technical Specifications

Parameter	Specification
Tissue Type	Reconstructed Human Pigmented Epidermis (RHPE)
Cell Types	Normal human keratinocytes (NHEK) + melanocytes (NHM)
Seeding Ratio	Keratinocyte : Melanocyte = 10:1
Culture Substrate	Inert polycarbonate filter (cell culture insert)
Culture Method	Air-liquid interface
Culture Medium	Chemically defined, serum-free (no TPA, no IBMX)
Culture Duration	10 days
Surface Area	0.5 cm^2
Available Phototypes	II (light / Caucasian), IV (intermediate), VI (dark)

Macroscopic Appearance

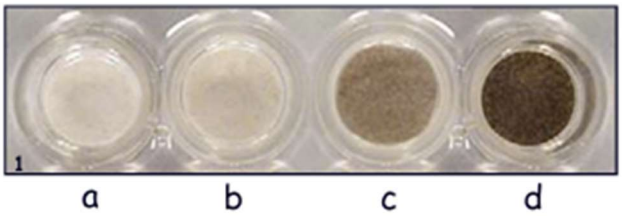

Fig 1. Tanning Degree. Macroscopic comparison of reconstituted human epidermis: (a) without melanocytes, and with melanocytes from phototype II (b), phototype IV (c), and phototype VI (d). The progressive darkening corresponds to increasing melanocyte donor skin phototype, demonstrating spontaneous melanogenesis without artificial stimulators.

Depigmentation Assay: Skin Care Cream Containing a Whitening Agent

1.2 µL of a formulation containing kojic acid is deposited daily onto the surface of the stratum corneum of pigmented epidermal tissues of phototype VI (0.63 cm² surface) for 3 days. 48 hours after the last application (at day 5), tissues are investigated by histological techniques.

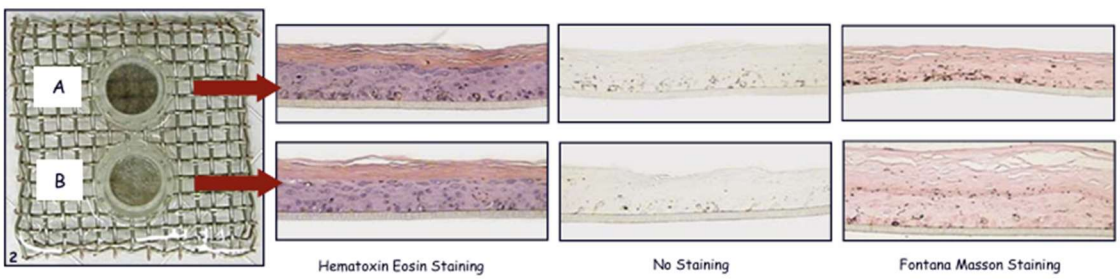

Fig. 2: Repeated applications of the skin care cream decrease the tanning degree (B) as compared to the untreated control (A). By Fontana Masson staining, a correlation between reduced pigmentation and lower melanin content present in the melanocytes is observed.

Pigmentation Assay: UV Irradiation and/or Chemical Modulators

Human pigmented epidermal tissues of phototype IV are exposed daily to UVB irradiation at 50 mJ/cm², then to UVA irradiation at 1 J/cm² for 3 days (from day 12 to day 14 of culture) using a Vilber Lourmat RMX 3W irradiator, and cultivated in defined medium supplemented with pigmentation stimulators such as Melanotan I (MT-I) or Adrenocorticotropic Hormone (ACTH) at different concentrations.

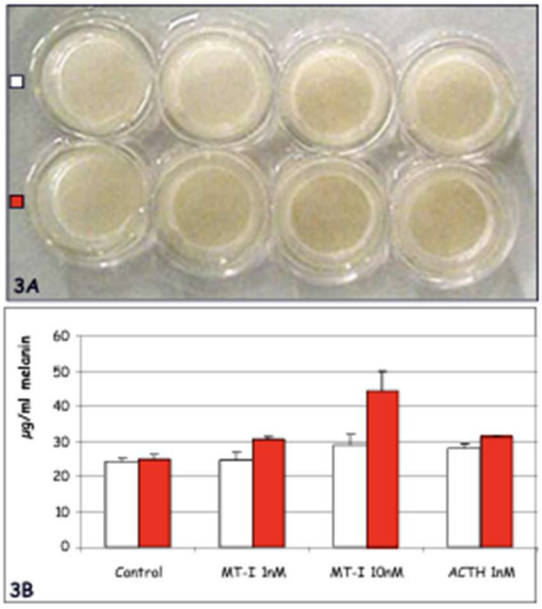

Fig. 3: Pigmentation is induced by UVA and UVB irradiation. The addition of MT-I and ACTH provokes an increase of pigmentation. These observations are confirmed by colorimetric melanin quantification performed after extraction with Soluene according to the Ozeki protocol.

Perspective: Full-Thickness Pigmented Skin Model

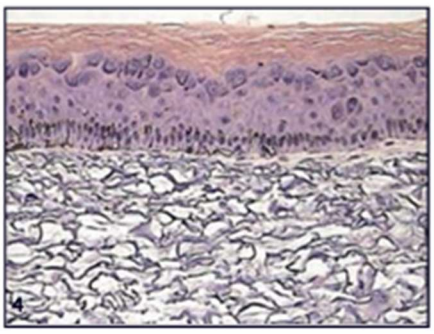

Fig. 4: Full-thickness skin model

By cultivating normal human fibroblasts in a dermal substrate, followed by co-cultivating human keratinocytes and melanocytes on the top of the dermal equivalent, a very well differentiated pigmented epidermis is obtained in which melanocytes are exclusively localized in the basal cell layer. This full-thickness model is currently under evaluation for testing modulators of pigmentation.

Conclusion

The SkinEthic™ RHPE human pigmented skin models reconstituted in vitro in chemically defined medium are excellent tools to evaluate the activity of new compounds on melanogenesis and to study their mechanisms in the skin's pigmentary system. The model supports both

depigmentation (whitening agents) and pigmentation (UV, hormonal stimulators) assays with quantifiable, objective endpoints.

For further information

Manufacturer	EpiSkin — L'Oréal Research & Innovation
Address	45 Rue St. Philippe, 06000 Nice, France
Website	www.episkin.com

References: Sahuc F., De Wever B. & Rosdy M. "Human Pigmented Epidermis Reconstituted In Vitro In Chemically Defined Medium Used For Evaluation Of Modulation Of Pigmentation." 12th Meeting of the European Society for Pigment Cell Research (ESPCR), September 22–25, 2004, Paris, France.

Intended Use

FOR RESEARCH PURPOSES ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES OR ANY OTHER HUMAN USES.

The SkinEthic™ RHPE human tissue model is standardized for in vitro testing of test chemicals or formulations applied on the surface of the tissues or in the medium underneath. In vitro testing applications include irritation, absorption, and pharmacological activity evaluation. The model is exclusively for in vitro use and not for use in human or animal experiments.

Quality Control & Safety

The manufacture of the kit follows procedures laid within a certified ISO 9001 Quality Management System. Media and models are prepared under aseptic conditions. Each batch of tissue is Quality Assured according to specific Quality Control (QC) standards tests. Results are reported in a "Technical Data, Safety Sheet and Certificate of Analysis" available on the Order website.

Human cells used to produce the tissue models have been tested for the absence of HIV, hepatitis B, hepatitis C, bacteria, and mycoplasma. Appropriate safety and sterility precautions must be taken while manipulating the human tissue models and media. It is strongly recommended to wear safety glasses, lab coat and gloves during handling and to work under a biohazard class II hood. Waste disposal: tissue models, material in contact with them and used media must be decontaminated and disposed as biological wastes. Material Safety Data Sheets (MSDS) are available on demand.

Shipping, Storage & Handling

Tissues are shipped at room temperature in a multi-well plate filled with an agarose-nutrient solution. The multi-well plates are sealed with a white tape and packed sterile in a plastic bag. SkinEthic Maintenance Medium (SMM) or SkinEthic Growth Medium (SGM) is shipped along with

the tissue models. A specific SGM for the RHPE model is available for sale (SGMRHPE). Products are delivered in accordance with international requirements for transportation.

Upon Arrival — Tissue Maintenance Protocol:

1. Remove the multi-well plate from the bag and strip off the tape.
2. Open the plate under a sterile airflow (recommended).
3. Carefully take out each insert containing the tissue model, remove any remaining agarose that adheres to the outer sides of the insert, and immediately place in a plate in which each well has previously been filled with medium at room temperature (see table below).
4. Make sure that the medium is never above the polycarbonate filter and that no air bubbles are formed underneath the insert.
5. Place tissues in the incubator at 37°C, 5% CO2, and saturated humidity.
6. Testing can be initiated after overnight incubation.

Insert Sizes & Medium Volume

	Small	Medium	Large	HTS 24	HTS 96
Plate for tissue storage	6-well plate	6-well plate	6-well plate	24-well HTS plate	96-well HTS plate
Volume / well	1 ml / day	2 ml / day	2 ml / day	0.6 ml / day	0.2 ml / day
Example plate reference	Falcon ref. 353046	Falcon ref. 353046	Falcon ref. 353003	Corning ref. 3524	Corning ref. 3383
For weekends	3 ml / 72h	8-12 mL / 72h	12 mL / 72h	/	/

Media Storage & Use

Media should be stored at 2-8°C and in the dark. The shelf-life is limited — please refer to the expiration date mentioned on the packaging. Use media at room temperature: do not pre-heat.

- To maintain tissues for 48 hours only, use SMM and change the medium after 24 hours.
- To maintain tissues for more than 48 hours, use SGM and change daily.
- Note: A specific SGM for the RHPE model is available (SGMRHPE).
- Act quickly as the tissues dry out rapidly when not in contact with medium.
- After the maintenance period, remove the medium and replace it with fresh medium before initiating testing.

Culture & Incubation Conditions

Parameter	Condition
Temperature	37°C
CO2	5%
Humidity	Saturated
Maintenance Medium (≤48h)	SkinEthic Maintenance Medium (SMM)
Growth Medium (>48h)	SkinEthic Growth Medium RHPE (SGMRHPE)
Media Storage	2-8°C, in the dark, do not pre-heat

EpiSkin — L'Oréal Research & Innovation — 4, rue Alexander Fleming — 69366 Lyon Cedex 07 — France
Phone: +33 (0)4 37 28 72 00 — Fax: +33 (0)4 37 28 72 28 — www.episkin.com