

MANUAL / epiCS®-M
*Human Epidermis Equivalent
with Melanocytes*

CELLSYSTEMS – YOUR PARTNER FOR OVER 20 YEARS.

We focus on primary and stem cells, organotypical 3D tissue models as well as on human bio specimens. 3D in vitro tissue models are developed and produced in our on-site high class cleanroom facilities. These also offer perfect conditions for tailored contract services in various application areas of in vitro toxicology and drug development. Combined with our strong application support, flexibility and a swift response time we are set up for your projects' needs.



CellSystems' epiCS-M is a human, in vitro reconstructed epidermis with melanocytes. This pigmented epidermis is a 3D co-culture system consisting of normal human epidermal keratinocytes and melanocytes cultured in cell culture inserts. This highly differentiated model of the human epidermis shows epithelial stratification and cornification. The melanocytes are located in the basal cell layers and undergo spontaneous melanogenesis during culture.

Description // epiCS-M

The three dimensional human epidermis equivalent epiCS-M is reconstructed from normal human primary epidermal keratinocytes and melanocytes. After culturing both cell types in cell culture inserts (0.6 cm²) under submerged conditions, the tissues are lifted to the air-liquid interphase to induce differentiation, epithelial stratification and cornification. The cellular structure of epiCS-M closely resembles a natural human epidermis showing a basement membrane, proliferating keratinocytes, melanocytes in the basal cell layers and a stratum corneum with an intact barrier function. Melanogenesis can be observed throughout the culture period. The pigmented epidermis model is available with melanocytes from Caucasian, Asian-Caucasian or Afro-American donors.

The production process of epiCS-M at CellSystems ISO 9001:2008 certified laboratories in Germany ensures highly standardized epidermis equivalents. epiCS-M can be cultured for up to 4 weeks and is supplied in the convenient 24-well format to facilitate topical application of test materials. This efficient and economical in vitro tool is the system of choice due to instant availability and high reproducibility.

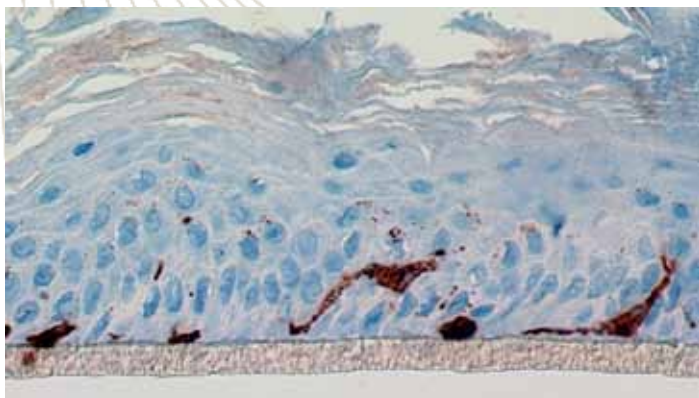


Figure 1 / Localisation of melanocytes in epiCS-M after 14 days of culture at air-liquid interphase. Staining with an anti-HMB45 antibody and peroxidase conjugated anti-mouse secondary antibody with AEC (3-amino-9-ethylcarbazole) as peroxidase substrate. Bar 10 µm.

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Main Applications //

The pigmented epidermis model epiCS-M is an ideal tool for skin bleaching or tanning studies as well as melanogenesis research. Studies can be carried out using melanin content or macroscopic darkening as endpoints. epiCS-M can be cultured for up to 4 weeks. This allows long term studies like efficacy testing of compounds or skin barrier formation and maturation. Test substances can be applied once or repeatedly onto the epidermis or into the culture medium.

SKIN WHITENING AND TANNING

The human skin like properties of epiCS-M allow long- or short-term experiments. These comprise systemically or topically applied test compounds or special culture conditions, e. g. exposition of the reconstructed epidermis to UV light, which affects the degree of pigmentation.

Standard endpoints are the visual appearance of the reconstructed skin, melanin content of each model and accompanying histological analysis. Protocol examples for tanning and whitening experiments can be found below.

Kit Contents //

The quantity of media and culture plates depends on the number of ordered epiCS-M. Single kit compounds can be purchased separately. See section "products".

- *epiCS-M epidermis equivalent (0.6 cm²) in 24-well transport plate*
- *epiCS-M Culture Medium*
- *epiCS-M MTT Assay Medium*
- *6-well culture plates*
- *Certificate of Analysis*

Handling Instructions //

ADDITIONALLY MATERIAL REQUIRED

- *Class II biological safety cabinet*
- *Incubator (37 °C, 5 % CO₂, 95 % humidity)*
- *Water bath (37 °C)*
- *Sterile pipette tips*
- *Sterile pair of tweezers*

Usage Note

// For research use only - not approved for human or veterinary use or for diagnostic or clinical procedures.

**PREPARATION ON RECEIPT**

Check the kit for completeness and potential transport damages.

The kit must be processed immediately as described below.

- - *Set up the 6-well plates and pipette 2 ml cold epiCS-M Culture Medium (4 °C to 8 °C) into each well.*
- - *Remove the Parafilm™ from the 24-well transport plate, containing the epidermis equivalents and open under sterile conditions.*
- - *Lift the inserts with a sterile pair of tweezers and transfer them into the sterile 6-well plate filled with epiCS-M Culture Medium. Make sure not to transfer any agarose.*
- - *Avoid air bubbles between inserts and the bottom of the culture dish by setting the inserts at an angle into each well.*
- - *Incubate the epidermis equivalents for at least 2 hours at 37 °C, 5 % CO₂, and 95 % humidity before performing first experiments.*
- - *After this adaptation, apply test material onto the stratum corneum or dissolved in medium.*
- - *Culture the epidermis equivalents in the incubator (37 °C, 5 % CO₂, 95 % humidity).*
- - *In case you intend to culture epidermis equivalents for more than 24 hours, change medium every other day. Remove old medium and replace it with 2 ml new epiCS-M Culture Medium (37 °C) per well.*
- - *Store epiCS-M Culture Medium and epiCS-M Assay Medium at 4 - 8 °C.*



Protocols //

SKIN TANNING WITH IBMX

3-Isobutyl-1-Methyl-Xanthine (IBMX) is known to stimulate melanogenesis. It is added directly into the culture medium. Experiments should start best after the 2 hours adaption phase.

- - Dissolve IBMX in sterile DMSO to get an appropriate stock solution (e.g. 50 mM) and store at 4 - 8 °C.
- - Make up a final IBMX concentration of 50 µM in epiCS-M Culture Medium. Ensure that the final DMSO concentration does not exceed 0.1 %.
- - Aspirate the regular epiCS-M Culture Medium and replace it with 2 ml freshly prepared medium containing IBMX (37 °C) for each well.
- - Culture the epidermis equivalents in the incubator (37 °C, 5 % CO₂, 95 % humidity).
- - Remove the old medium and replace it with 2 ml new epiCS-M Culture Medium containing IBMX (37 °C) for each well. Change medium every other day up to 2 weeks.

SKIN WHITENING WITH KOJIC ACID

Kojic Acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone) is a known inhibitor of melanogenesis and therefore often used for whitening in cosmetic products. In contrast to the tanning experiment described above, Kojic Acid is applied topically onto the epiCS-M surface. This whitening experiment is started 3 to 5 days after receipt of the epidermis equivalents.

- - Prepare 2 % Kojic Acid solution.
- - Apply 25 µl of Kojic Acid directly onto the surface of the epidermis equivalent.
- - Culture the epidermis equivalents in the incubator (37 °C, 5 % CO₂, 95 % humidity).
- - Repeat this after every routine medium change (every other day) up to 2 weeks.

Information

// There are several substances which are known to stimulate or inhibit melanogenesis. The protocols on the left are examples for two of these active chemicals.

Other substances may require changes of the protocols.



MELANIN MEASUREMENT

The measurement of total melanin content, e.g. after application of melanogenesis stimulators or inhibitors, is described below.

Required Material /

- - *Solvable™ (Perkin Elmer)*
- - *Synthetic melanin standard*
- - *Pointed, bent pair of tweezers*
- - *96-well plate (flat bottom)*
- - *Cell culture insert membrane without cells as control*
- - *Water bath*
- - *Floater*
- - *Sharp scalpel*
- - *Reaction tubes (1.5 ml)*
- - *Fine pipette 1000 µl*
- - *Spectrophotometer (wavelength = 492 nm)*

Melanin Extraction and Measurement /

- - *Prepare reaction tubes filled with 1 ml Solvable™.*
- - *Cut out the insert membrane with the epidermis equivalent from the cell culture insert using a sharp scalpel and transfer it into one of the prepared reaction tubes.*
- - *Use one cell culture insert membrane without cells as control.*
- - *Incubate the tubes in boiling water for 60 minutes using a floater.*
- - *Dispense duplicates of 100 µl of each sample dilution to an appropriate well of a 96-well flat bottom plate and read the absorbance at 492 nm using a spectrophotometer.*
- - *To get absolute concentrations use synthetic melanin as standard. The standard curve should range from 0 - 200 µg/ml.*



MTT VIABILITY ASSAY

The MTT viability assay is a colorimetric assay for measuring the activity of enzymes that reduce MTT to formazan dyes.

Required Material /

- MTT reagent (Thiazolyl Blue Tetrazolium Bromide)
- epiCS-M MTT Assay Medium
- 24- and 96-well plates (flat bottom)
- Isopropanol
- Fine pipette 1000 µl
- Spectrophotometer (wavelength = 540 - 570 nm)

MTT Extraction and Measurement /

- Prepare a 1.0 mg/ml solution of MTT in epiCS-M MTT Assay Medium. Add 300 µl to each well of a new 24-well plate.
- Take each insert with tweezers, remove excess culture medium by dabbing the bottom on a paper towel and transfer it quickly into one well of the prepared 24-well plate.
- Incubate 24-well plates for 3 hrs (37 °C, 5 % CO₂, 95 % humidity)
- Take each insert with tweezers, remove excess MTT medium by dabbing the bottom on a paper towel and transfer the inserts into a new 24-well plate.
- Add 2 ml isopropanol directly to each insert. The insert in the well should be submerged completely.
- Wrap the plate with parafilm and shake it for 2 hours at room temperature on a vertical shaker or store at 4 - 8 °C overnight.
- Puncture the insert membranes with an injection needle (~ gauge 20, ~ 0.9 mm diameter) and allow the extract to run into the well from which the insert was taken. Discard the insert.
- Carefully shake on a vertical shaker for about 10 min.
- Dispense duplicates of 200 µl of each sample to a 96-well flat bottom plate and read the absorbance at 540 – 570 nm using a spectrophotometer.
- Viability is calculated as follows: $\text{Viability (\%)} = (\text{absorbance treated models} / \text{absorbance untreated control models}) \times 100$

MTT Assay

// The reduction of tetrazolium salts is widely accepted for the measurement of cell viability or cell proliferation.

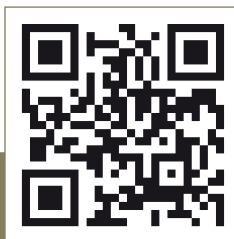
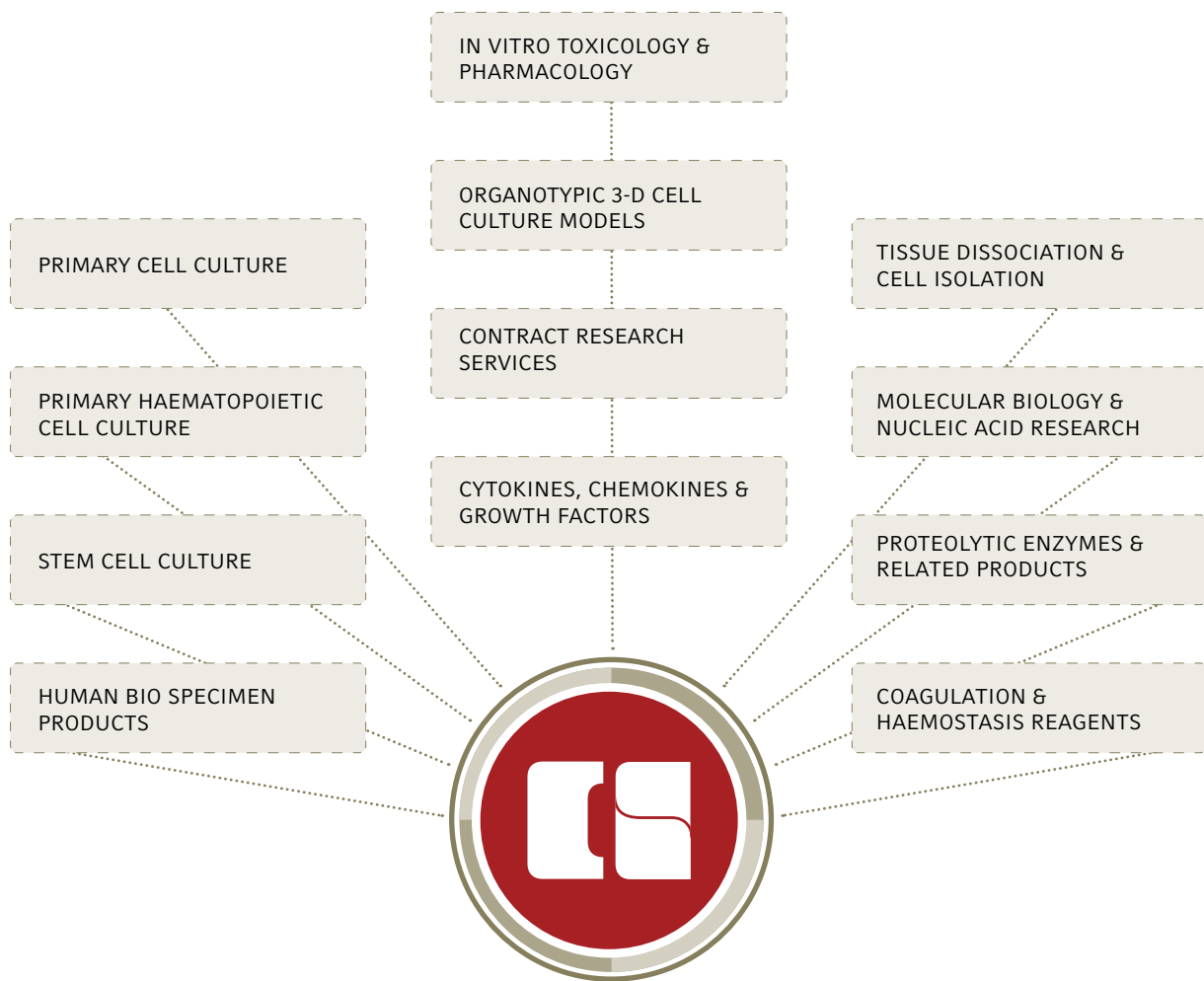
The yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectroscopic methods.

**Products //**

epiCS-M AND RELATED PRODUCTS			
Product Name	Description	Size	Cat.-No.
epiCS-M Caucasian donor	Human epidermis equivalent with melanocytes from Caucasian donors, epiCS-M Culture Medium and epiCS-M MTT Assay Medium included	1 pcs	CS-1101
epiCS-M Asian-Caucasian donor	Human epidermis equivalent with melanocytes from Asian-Caucasian donors, epiCS-M Culture Medium and epiCS-M MTT Assay Medium included	1 pcs	CS-1111
epiCS-M Afro-American donor	Human epidermis equivalent with melanocytes from Afro-American donors, epiCS-M Culture Medium and epiCS-M MTT Assay Medium included	1 pcs	CS-1121
epiCS-M Culture Medium	For epiCS-M culture	125 ml	CS-3151
epiCS-M Culture Medium	For epiCS-M culture	500 ml	CS-3152
epiCS-M MTT Assay Medium	Solvent for MTT used for MTT viability assays with epiCS-M	25 ml	CS-3130
epiCS-M MTT Assay Medium	Solvent for MTT used for MTT viability assays with epiCS-M	50 ml	CS-3131
Nylon Meshes	For improving the contact between compound and epiCS-M surface	12 pcs	CS-5010







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