

MANUAL / epiCS®
Human Epidermis Equivalent

CELLSYSTEMS – YOUR PARTNER FOR OVER 20 YEARS.

We focus on primary and stem cells, organotypical 3D tissue models as well as on diseased cells and tissues. 3D in vitro tissue models are developed and produced in our on-site high class cleanroom facilities. These also offer perfect conditions for tailor-made 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.

www.cellsystems.de

For more information on reconstructed human epidermis:

www.skininvitro.com



CellSystems' epiCS is a human, in vitro reconstructed 3D epidermis, consisting of normal human epidermal keratinocytes cultured in cell culture inserts. This highly differentiated model of the human epidermis shows epithelial stratification and cornification.

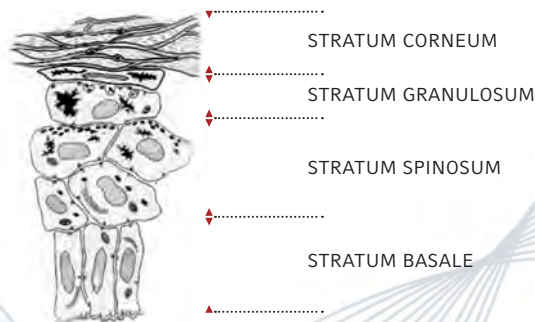
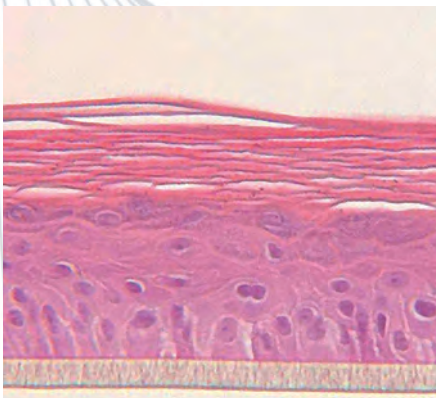
Description // epiCS

The three dimensional human epidermis equivalent epiCS is reconstructed from normal human primary epidermal keratinocytes. After culturing the keratinocytes 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 closely resembles natural human epidermis showing a basement membrane, proliferating keratinocytes and a stratum corneum with an intact barrier function. Furthermore, it shows an excellent in vitro / in vivo comparability.

The production process of epiCS at CellSystems® ISO 9001:2008 certified laboratories in Germany ensures highly standardized epidermis equivalents. epiCS 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 economic alternative to animal testing is the system of choice due to instant availability and convenient use.

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Main Applications //

REGULATORY TOXICOLOGY

Due to legal restrictions on animal testing, three dimensional in vitro models are increasingly used for regulatory toxicology or to measure effects of skin-active materials. The epidermis equivalent epiCS is an ideal tool for testing pharmaceutical and chemical substances. Different effects of test compounds can be determined by multiple end-point analysis including, but not limited to, gene expression, viability, histology and cytokine release.

Main applications for epiCS are skin corrosion, skin irritation, skin sensitisation testing as well as phototoxicity or genotoxicity studies in the context of regulatory toxicology or research.

epiCS is validated for the classification of compounds according to the OECD test guideline 431: "In Vitro Skin Corrosion: Human Skin Model Test". The European Centre for the Validation of Alternative Methods (ECVAM) has accepted this method to be used for distinguishing between corrosive and non-corrosive chemicals. ECVAM has published the ESAC statement on the scientific validity of the epiCS method (name change from EST1000 to epiCS in 2012) for skin corrosion testing (June, 12th, 2009).

epiCS can be used for sub-categorisation of corrosive chemicals (see OECD Test Guideline 431 (2014)).

Furthermore, epiCS has successfully undergone a multicentre validation study for in vitro skin irritation testing according to OECD Test Guideline 439. The corresponding ESAC opinion is expected soon, the citation in the OECD Test Guideline 439 in the next guideline version.

Kit Contents //

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

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

Usage Note

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



Handling Instructions //

ADDITIONAL MATERIAL REQUIRED

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

PREPARATION ON RECEIPT

Check the kit for completeness and potential transport damages.
The kit must be processed immediately as described in this protocol.

- - Set up the 6-well plates and pipette 1 ml cold epiCS Culture Medium (4 - 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 sterile pair of tweezers and transfer them into the sterile 6-well plate filled with epiCS 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 4 hours at 37 °C, 5 % CO₂, and 95 % humidity before performing first experiments.
- - After this adaptation, apply test compounds topically onto the stratum corneum or dissolved in culture medium.
- - Culture the epidermis equivalents in the incubator (37 °C, 5 % CO₂, 95 % humidity).
- - In case you intend to culture the epidermis equivalents for more than 24 hours, change medium every day. Remove medium and replace it with 1 ml new epiCS Culture Medium (37 °C) per well.
- - Store Culture Medium and MTT Assay Medium at 4 - 8 °C.

Biohazard Note

// Although the cells are tested negative for HIV-1, HBV, HCV, mycoplasma, bacteria and fungi, no test method can guarantee the absence of known and unknown infectious agents. Consequently, all products of human origin should always be considered potentially biohazardous and appropriate precautions should be taken. Use good laboratory practice and aseptic technique at all times.



Protocols //

IN VITRO SKIN CORROSION TESTING (OECD TG 431)

epiCS is validated for the classification of compounds according to the OECD test guideline 431 (see section "Main Applications") and can be used for sub-categorisation of corrosive chemicals (OECD Test Guideline 431, 2014).

To evaluate the skin corrosive potential of a given compound the viability of the epidermis equivalents is determined after exposure to the test compound and compared to untreated controls. A detailed SOP for in vitro skin corrosion testing with epiCS can be downloaded from www.skininvitro.com (see "Resource Center").

IN VITRO SKIN IRRITATION TESTING (OECD TG 439)

The epiCS Skin Irritation Test method has successfully been validated according to OECD Test Guideline 439. A detailed SOP for epiCS skin irritation testing (epiCS SIT) can be downloaded from www.skininvitro.com (see "Resource Center").

IN VITRO SKIN SENSITISATION TESTING

Currently, epiCS undergoes a multicentre pre-validation study for skin sensitisation testing.

It was shown that epiCS exposed to skin sensitisers release significant higher levels of IL-18 than epiCS exposed to non-sensitisers, allowing to discriminate sensitisers from non-sensitisers. It was also observed that strong sensitisers reduce epiCS viability much more than weak sensitisers. In combination, IL-18 and viability measurement of epiCS will therefore identify sensitisers and rank them to their sensitising potency. When compared to data generated with the LLNA assay, high correlation was observed.

PHOTOTOXICITY TESTING

The core test for evaluating the skin phototoxic potential of a compound is the BALB 3T3 neutral red uptake phototoxicity test (3T3 NRU, OECD TG 432). However, this test has limitations. Hydrophobic chemicals can be tested only at low concentrations due to their lack of aqueous solubility. Also many complex mixtures or formulations cannot be tested. To cope with these limitations, the use of reconstructed epidermis equivalents is a useful follow-up test.

Application of Test Sample /

For statistical reasons, the assay should be carried out in triplicates. Test compounds as well as control compounds are applied to the epidermis equivalents followed by UV-irradiation (n=3) or no UV-irradiation (n=3).

Information

// Skin corrosion refers to the production of irreversible tissue damage in the skin following the application of a test material (as defined by the Globally Harmonised System for the Classification and Labelling of Chemical Substances and Mixtures (GHS)).

Skin irritation refers to the production of reversible damage to the skin following the application of a test chemical for up to 4 hours (as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS)).



Hence, for each test and for each control compound 6 epidermis equivalents are required.

- - Apply 30 μ l of the test compound, positive control compound (e.g. chlorpromazine 0.01 %), and negative control compound (e.g. 1 x PBS) topically (to the surface of the epidermis equivalent) with a sterile micro-pipette.

Irradiated Epidermis Equivalents /

- - Irradiate 3 epidermis equivalents (one triplicate per test compound or control) with 6 J/cm² UVA.

Non-Irradiated Epidermis Equivalents /

- - In parallel the corresponding 3 epidermis equivalents (serving as non-irradiated control tissues, one triplicate) are stored in the dark.
- - Remove the test compounds from the surface of the epidermis equivalents by rinsing the tissues thoroughly using a squeeze water bottle with sterile PBS for at least twenty times followed by waving them in a beaker filled with 1 Litre PBS. Ensure that the epidermis equivalents are free of test compound.
- - Remove excess PBS by gently shaking the insert and dab the bottom on a piece of paper towel.
- - Incubate all epidermis equivalents at 37°C, 5 % CO₂ and 95 % humidity for 18 hrs.
- - Perform a standard MTT Assay (see below) to determine the viability of the epidermis equivalents.

MTT VIABILITY ASSAY

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

Required Material /

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

MTT Assay

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

The yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium 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.

**MTT Extraction and Measurement /**

- - Prepare a 1 mg/ml solution of MTT in epiCS MTT Assay Medium. Add 300 μ l 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 hrs 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 μ l 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: $Viability (\%) = (absorbance \text{ treated models} / absorbance \text{ untreated control models}) \times 100$

PREPARATION OF CRYOSECTIONS

Cryosections allow rapid microscopic analysis.

Required Material /

- - 24-well plate
- - Pair of tweezers
- - Fixation solution: 8 % formaldehyde in 200 mM HEPES - buffer pH 7.3
- - Tissue freezing medium
- - Embedding molds
- - Liquid nitrogen
- - Coated slides
- - Cryo-microtome
- - Fine pipette 1000 μ l
- - Pasteur pipette
- - Sharp scalpel



Fixation /

- - Place the inserts into a 24-well plate.
- - Cover carefully the inserts with 1.5 ml fixation solution.
- - Incubate at least for 30 min at room temperature.
- - Aspirate the fixation solution from top of the tissue using a Pasteur pipette.
- - Wash 3 times for 5 min with 2 ml of PBS each. Remaining fixation solution is washed away by this treatment.
- - Cut the membrane from the bottom of the insert using a sharp scalpel.

Embedding and Cutting /

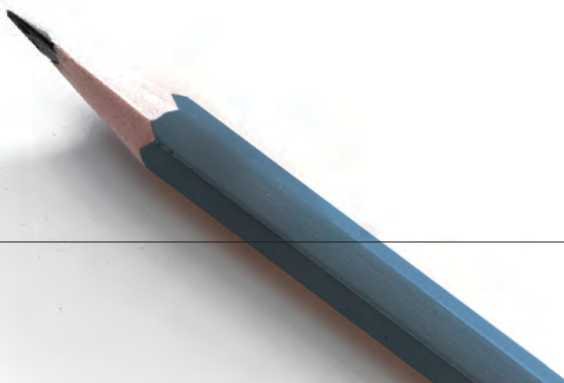
- - Embedding is done in suitable molds with embedding solution.
- - Fill a suitable container (Styrofoam, plastic safe for freezing, dewar) with liquid nitrogen.
- - Hold the embedding mold with a pair of tweezers at one edge and hold it into the gaseous phase of the liquid nitrogen until the embedding medium is homogenously white. This leads to slow freezing and therefore reduced forming of ice crystals.
- - Remove the embedded tissues from the embedding molds.
- - Cut at -30 °C with a cryo-microtome to 5 - 10 µm slides.
- - Collect cuts on coated slides and dry them for 10 min at 40 °C.

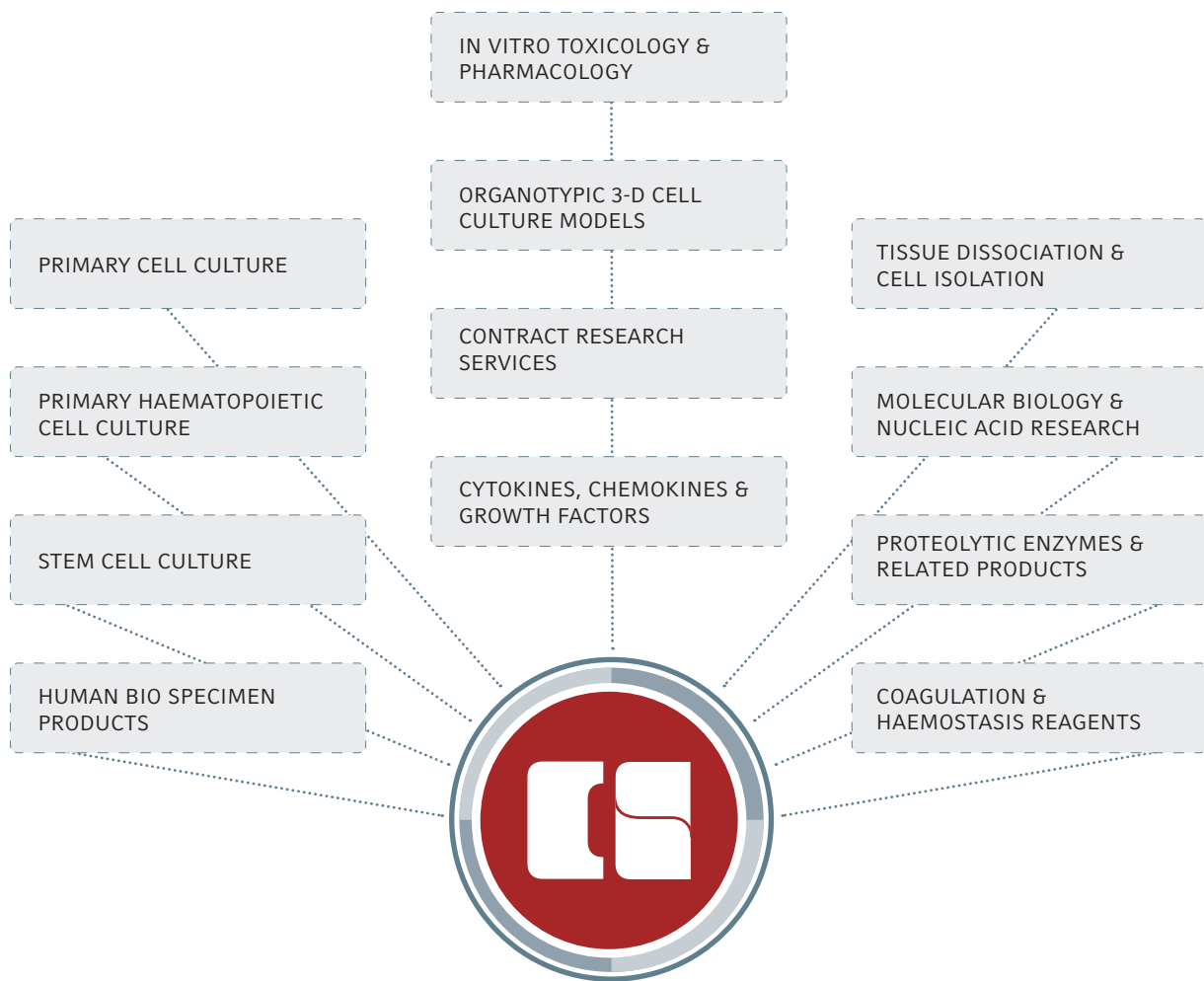


Products //

epiCS AND RELATED PRODUCTS			
Product Name	Description	Size	Cat.-No.
epiCS	Human epidermis equivalent, epiCS Culture Medium and epiCS MTT Assay Medium included	1 pcs	CS-1001
epiCS Validation Study Corrosion ^{1/}	Registration for first participant from a laboratory	1 Kit	CS-1018-VC
epiCS Validation Study Corrosion – Additional Participant ^{1/}	Registration for one additional participant from a laboratory	1 Kit	CS-1009-VC
epiCS Validation Study Irritation ^{1/}	Registration for first participant from a laboratory	1 Kit	CS-1018-VI
epiCS Validation Study Irritation – Additional Participant ^{1/}	Registration for one additional participant from a laboratory	1 Kit	CS-1009-VI
epiCS Culture Medium	For epiCS culture	50 ml	CS-3050
epiCS Culture Medium	For epiCS culture	75 ml	CS-3051
epiCS Culture Medium	For epiCS culture	100 ml	CS-3052
epiCS Culture Medium	For epiCS culture	125 ml	CS-3053
epiCS MTT Assay Medium	Solvent for MTT used for MTT viability assays with epiCS	25 ml	CS-3030
epiCS MTT Assay Medium	Solvent for MTT used for MTT viability assays with epiCS	50 ml	CS-3031
Nylon Meshes	For improving the contact between compound and epiCS surface	12 pcs	CS-5010

^{1/} epiCS Validation Study Corrosion and epiCS Validation Study Irritation are inter laboratory studies to confirm reproducibility and reliability of results for skin corrosion and skin irritation testing of compounds with epiCS in your laboratory. The corrosive or irritative potential of blinded test substances are determined simultaneously in your facility and CellSystems' DIN ISO 9001:2008 certified laboratories.





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