PRODUCT SPECIFICATION

instaCELL KeratinoSens® Assay Kit

CatN°: SF220-01
Lot#: KS-20220104
Expiry Date: 06.04.2023

PRODUCT DEFINITION

Test kit to assess the skin sensitization potential of chemicals and leachables by their application to cultures of KeratinoSens® cells and the subsequent determination of cell viability.

QUALITY SPECIFICATION OF THE CELLS

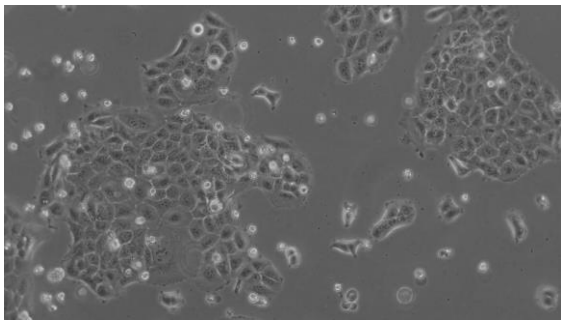
	Batch Quality Control	Specification Limits
Cell Count	**2.36E+06**	2.2E+06<>2.8E+06
Homogenity (cell count)	**94%**	≥ 90%
Viability (after thawing)	**98%**	≥ 90%
Proliferative Capacity	**97%**	≥ 70%
Debris/Cell Ratio	**0.2**	≤ 1.0
Aggregation	**0.1**	≤ 2.0
Sterility (bacteria, yeast, fungi)	**passed**	negative after 7 days
Sterility (mycoplasma)	**passed**	negative by PCR
Morphology	**passed**	unalterd to reference
EC1.5 DNCB	**4.1 µM**	≤12.5 µM
EC1.5 EGDMA	**54.2 µM**	30 - 100 µM
EC1.5 Lactic Acid	**>1000 µM**	≥ 1000 µM

KIT CONTENT

	Lot#	Storage
Recovery Buffer H	**91-211209NR01**	**-20°C**
Assay Buffer H	**91-210429NR01**	**-20°C**
Assay Medium H	**91-211204NR01**	**-20°C**
Positve Control (25mM EGDMA)	**91-211209NR02**	**-20°C**
Resazurin Solution	**91-210604NR01**	**-20°C**
Promega, OneGlo™	**461429**	**-20°C**
Assay Ready KeratinoSens Cells	**92-191122JP03**	**< -140°C**
96-well Assay Plate	**E20103EA**	**RT**

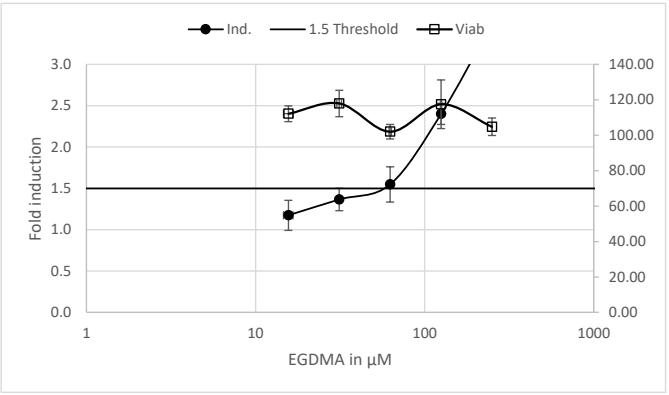
Sterility was analyzed by microscopic/visual control after seven days according to sterility testing. Functionality of the content was tested by performing the assay with all listed batches.

MORPHOLOGY:



Morphology of KeratinoSens Assay Ready Cells

KERATINOSENS ASSAY:



Dose response of positive control Ethylenglykoldimethacrylat (EGDMA) performed according to the assay protocol. Fold-Induction and Viability

METHOD

Cell Viability Parameters: Viability parameters (viable cell count after thawing, grade of aggregation, percentage of debris) were determined from a pooled sample (SOP-2015-02). Briefly, assay ready cells were thawed in a water bath. 100 µl of each sample were pooled, diluted 1:1000 in CASY Ton buffer and measured (3 replica) in a CASY TT automatic cell counter. Vial to vial variation was determined in a plate-based viability assay.

Proliferative Capacity: Proliferative Capacity compares mean growth rate (T0 - T72 hours) of all sample vials with mean growth rate of exponentially growing culture. Freshly thawed cells from the assay ready cell samples were seed in a 96-well plate (3 replica each) according their specific 3 day seeding density. After 72 hours of cultivation, the proliferation of the cells was determined by addition of a metabolic cell dye (Resazurin) (SOP-2017-03).

Sterility Testing: Assay ready cells were seed in two specific bacteria growth brothes (Tryptic Soy Broth for aerob and Thioglycollate broth for anaerob conditions) and cultivated over a course of 14 days. After day 1, 4, 7 and 14 the cultures were analyzed microscopically for cell growth, cell morphology, and incidences of contamination (bacteria, yeast, or fungi). For mycoplasma testing from a three days old, sub-confluent culture 500 µl of the supernatant was taken and analyzed by PCR using a Mycoplasma detection kit (Minerva). Assay was performed according to the manufacturer protocol (SOP-2015-06).

Functional Testing: Assay Ready Cells were thawed, washed once in 10ml assay medium and seeded into a 96-well plate at 10.000 cells/well. Incubation for 24 hours at 37 °C and 5 % CO_2. The supernatant was discarded after adherence of the cells and replaced by serial dilutions of the reference chemicals DNCB, EGDMA and Lactic Acid. After 48 h of incubation at 37 °C and 5 % CO_2, 50µl of OneGlo™ (Promega), a luciferase substrate was added to each well. After 20 min, the luminescence was measured with an integration time of 1 s/well in a multiplate reader.

LIMITED USE

The product is provided under the terms of a limited use license provided with the kit. By breaking the sealed bag, the user is explicitly accepting the terms for limited use. The cell line uses Luciferase technology from Promega (U.S. Pat No. 8008006 & EU Pat. No. 1341808B1). The Kit may only be used under the terms of a limited use license which is attached as part of this kit.

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