instaCELL KeratinoSens® assay kit (Multiplex)

Product Information

Cat N° SF220-01

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Content

1 Description

The KeratinoSens® assay is a validated *in vitro* method to determine the skin sensitizing potential of a given test substance (OECD Test N°. 442D)[1]. In a recombinant HaCaT[5] derived keratinocyte cell line, the expression of a luciferase reporter gene (Promega) under control of the ARE antioxidant response element is measured. The Keap1-Nrf2-ARE pathway, which is a major regulator of cyto-protective responses to electrophile and oxidative stress, is considered to be the "Key Event 2" in the adverse outcome pathway of skin sensitization. The cell line and the assay (DB-ALM Protocol n°155[2]) have been developed by Emter & Natsch at Givaudan as a replacement for animal testing[3,4].

Figure 1: instaCELL KeratinoSens® assay kit

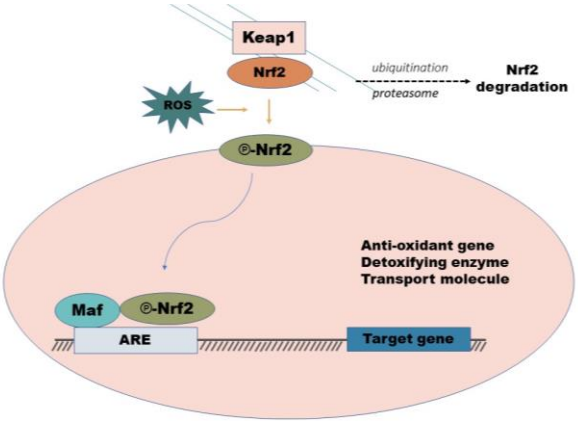

Figure 2: Keap1-Nrf2-ARE Pathway

The instaCELL KeratinoSens® assay kit includes prequalified Assay Ready Cells as well as media, reagents, controls, and plates to perform the assay according to the DB-ALM protocol n° 155 Annex 3. Assay Ready Cells are frozen aliquots of cells and can be used in the assay without prior cultivation, basically like a reagent. The technical proficiency of the instaCELL kit and Assay Ready Cells has been demonstrated with ten substances for which the correct classification as skin sensitizer could be determined according to OECD TG 442D.

Proficiency Substances	OECD Prediction	instaCELL Prediction	OECD $EC_{1.5}$ (µM)	instaCELL $EC_{1.5}$ (µM)	OECD IC_{50} (µM)	instaCELL IC_{50} (µM)
Salicylic Acid (non-sensitizer)	negative	negative	>1000	>1000	>1000	>1000
Lactic Acid (non-sensitizer)	negative	negative	>1000	>1000	>1000	>1000
Glycerol (non-sensitizer)	negative	negative	>1000	>1000	>1000	>1000
Isopropanol (non-sensitizer)	negative	negative	>1000	>1000	>1000	>1000
EGDMA (weak)	positive	positive	5 - 125	53	>500	>500
Cinnamyl alcohol (weak)	positive	positive	25 - 175	121	>1000	>1000
MBT (moderate)	positive	positive	25 - 250	107	>500	383
Metol (strong)	positive	positive	<12.5	4.1	20-200	20.4
MDBGN (strong)	positive	positive	<20	17.3	20-100	46.4
DNCB (extreme)	positive	positive	< 12.5	2.3	5 - 20	7.8

Table 1: Technical proficiency of the instaCELL KeratinoSens® assay kit according to OECD 442D[1]

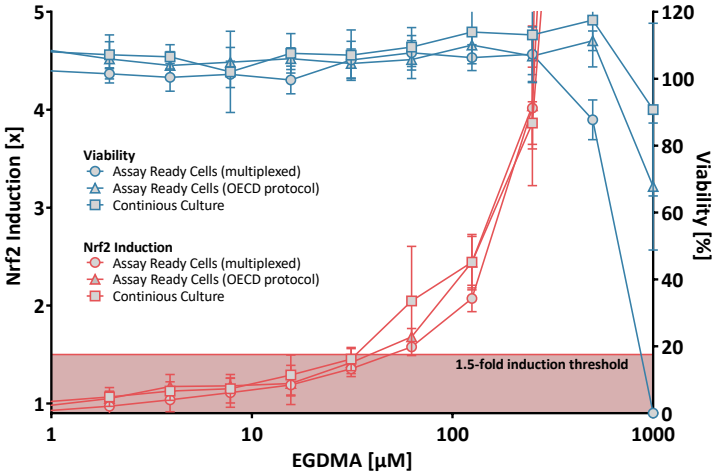

Figure 3: KeratinoSens® assay for Ethylene-glycol-dimethyl-acrylate (EGDMA). Result shall be interpreted by the consideration of cell viability (blue) and the induction of Nrf2 pathway by luciferase expression (red). A substance is considered a sensitizer when at any given concentration the fold induction of the pathway is greater 1.5 while the viability is still greater than 70%. The KeratinoSens® assays has been performed with cells from a continuous culture (☐) and Assay Ready Cells following the original OECD protocol[1] (△) or the multiplexed approach of DB-ALM protocol 155 Annex 3[2,4] (○).

2 Cell Information:

Originator:	Givaudan
Cell Type:	Reporter Cell Line
Tissue:	Keratinocytes (Skin)
Species:	human
Morphology:	Epithelial
Growth:	Adherent
Host Cell Line:	HaCaT[5]
Biosafety Level:	1
Recombinant Construct:	Luciferase reporter gene under transcriptional control of the ARE antioxidant response element from human AKR1C2 (Aldo-Keto Reductase family 1 member C2) in pGL4.17 [luc2/Neo] (Promega).

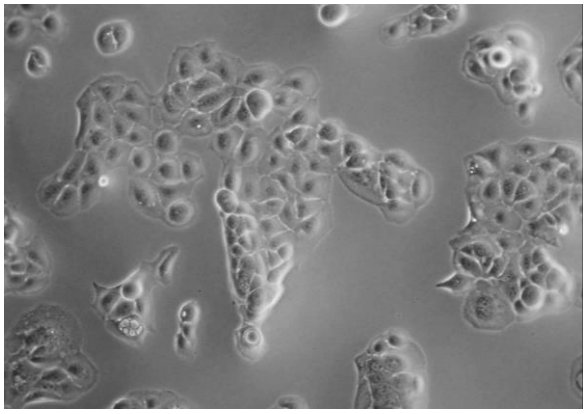

Figure 4: Morphology of KeratinoSens® Assay Ready Cells

3 Kit Content

• KeratinoSens® Assay Ready Cells	2 vial (1.25E6 cells)	RE242K
• Recovery Buffer H	2 bottle (10ml)	MD178-01
• Assay Medium H	1 bottle (60ml)	MD478-06
• Assay Buffer H	2 bottle (60ml)	MD378-06
• 96-well white, clear-bottom plates	2 plates	ZG02-09
• EGDMA, 25mM (Control)	2 vial (0.8ml)	RX507-01
• Resazurin Viability Assay	1 bottle (5ml)	RX718-01
• OneGlo™ Luciferase Assay System (Promega)	1 kit (10ml)	KR005-04
• Sealing tape	2 tapes	ZG12-03

Additionally required but not provided with the kit:
DMSO, DPBS, 2x 96-well plate, 50ml centrifuge tube.

4 Protocol of Use

4.1 day I: preparation of cells

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate all media and buffer to 37 °C.
- Thaw one vial of Assay Ready Cells in a water bath at 37 °C for 2 min. Prepare 9 ml of recovery buffer in a 50 ml centrifuge tube. Dispense the cells completely into the prepared tube.
- Centrifuge for 3 min at 200 x g and carefully aspirate the supernatant. Resuspend the cell pellet in 15 ml of assay medium. Dispense 125 µl of the cell suspension into each well of the provided assay plates except the wells reserved for blank values.
- Incubate for 24 h in a humidified incubator at 37 °C and 5 % CO_2.

4.2 day II: preparation of test chemicals and incubation with cells

- Dissolve the test chemicals in DMSO to a stock concentration of 200 mM.
- Prepare serial dilutions from the stock solutions in DMSO, to obtain twelve master concentrations of the test chemicals and five of the provided positive control. Dilute the master concentrations 25-fold into assay buffer.
- Aspirate medium from the assay plates and dispense 150 µl of assay buffer into each well.
- Add 50 µl of the diluted test chemicals and controls to the corresponding wells of the assay plates. Use assay buffer, containing 1 % DMSO, as solvent control.
- Cover the plate with sealing tape and incubate for 48h in a humidified incubator at 37 °C and 5 % CO_2.

4.3 day IV: staining and read-out

- Add 20 µl of resazurin to each well and incubate for 4 h at 37 °C.
- Measure the fluorescence on a plate reader at 540_{Ex} / 590_{Em} to determine the viability of the cells.
- Equilibrate all One-Glo™ components to room temperature. Reconstitute the One-Glo™ Reagent by adding 10 ml of the Luciferase Assay Buffer to the Luciferase Assay Substrate.
- Aspirate the supernatant of each well. Wash the cells once with 100 µl DPBS.
- Dispense 50 µl of DPBS and 50 µl of One-Glo™ reagent to each well and incubate for 20 min at room temperature in the dark.
- Measure luminescence with an integration time of 1s / well.

5 Analysis and Assay Acceptance Criteria

Use the instaCELL KeratinoSens Assay Evaluation sheet for the analysis of the assay (see section 8).

- Dose-dependent increase in luciferase induction, at least 2-fold above the solvent control.
- An $EC_{1.5}$ between 30-100 µM for the positive control.

6 Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	comp. 1 C12	comp. 1 C11	comp. 1 C10	comp. 1 C9	comp. 1 C8	comp. 1 C7	comp. 1 C6	comp. 1 C5	comp. 1 C4	comp. 1 C3	comp. 1 C2	comp. 1 C1
B	comp. 1 C12	comp. 1 C11	comp. 1 C10	comp. 1 C9	comp. 1 C8	comp. 1 C7	comp. 1 C6	comp. 1 C5	comp. 1 C4	comp. 1 C3	comp. 1 C2	comp. 1 C1
C	comp. 1 C12	comp. 1 C11	comp. 1 C10	comp. 1 C9	comp. 1 C8	comp. 1 C7	comp. 1 C6	comp. 1 C5	comp. 1 C4	comp. 1 C3	comp. 1 C2	comp. 1 C1
D	comp. 2 C12	comp. 2 C11	comp. 2 C10	comp. 2 C9	comp. 2 C8	comp. 2 C7	comp. 2 C6	comp. 2 C5	comp. 2 C4	comp. 2 C3	comp. 2 C2	comp. 2 C1
E	comp. 2 C12	comp. 2 C11	comp. 2 C10	comp. 2 C9	comp. 2 C8	comp. 2 C7	comp. 2 C6	comp. 2 C5	comp. 2 C4	comp. 2 C3	comp. 2 C2	comp. 2 C1
F	comp. 2 C12	comp. 2 C11	comp. 2 C10	comp. 2 C9	comp. 2 C8	comp. 2 C7	comp. 2 C6	comp. 2 C5	comp. 2 C4	comp. 2 C3	comp. 2 C2	comp. 2 C1
G		PC C5	PC C4	PC C3	PC C2	PC C1	PC C5	PC C4	PC C3	PC C2	PC C1	
H	SC	SC	SC	SC	SC	SC	PC C5	PC C4	PC C3	PC C2	PC C1	

test substance 1 (triplicates of 12 dilutions), test substance 2 (triplicates of 12 dilutions), positive control (triplicates of 5 dilutions), solvent control.

7 Stability & Storage

Performance of the kit is guaranteed within the specifications as defined in the certificate of analysis only before the expiration date as indicated on the packaging and only if stored and handled according to the instruction of this datasheet.

- Store Assay Ready Cells below -140 °C (e.g., in vapor phase of liquid nitrogen).
- Store all other reagents and media at -20 °C.

8 Literature & Related Documents

1. **OECD Test N°. 442D**: In Vitro Skin Sensitization
2. **DB-ALM Protocol N° 155**: KeratinoSens
3. **Emter R**, Ellis G, Natsch A. Performance of a novel keratinocyte-based reporter cell line to screen skin sensitizers in vitro. Toxicol Appl Pharmacol. 2010 Jun 15;245(3).
4. **Emter R**, Natsch A. A fast Resazurin-based live viability assay is equivalent to the MTT-test in the KeratinoSens assay. Toxicol In Vitro. 2015 Jun;29(4).
5. **Boukamp P**, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE.J Cell Biol. 1988 Mar;106(3):761-71
6. instaCELL KeratinoSens assay kit evaluation sheet
7. SF220 KeratinoSens® Assay Kit

9 Support

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10 Disclaimer

The cell line uses the Luciferase technology from Promega (U.S. Pat. No. 8008006 & EU Pat.No. 1341808B1). The cells may only be used under the terms of a limited use license which is attached as part of this kit.

The product is sold under the terms of a Limited Use Label License attached to the product. By breaking the seal of the product package, the user explicitly agrees to the license terms. Assay Ready Cells are for immediate assay use only. The user shall not propagate, passage, or refreeze the cells.

This product is intended for research use only. Do not use for diagnostic or therapeutic purposes.