KeratinoSens®

Assay Ready Cells

Certificate of Analysis

Lot-N°: 92-191122JP03

BATCH SPECIFICATIONS

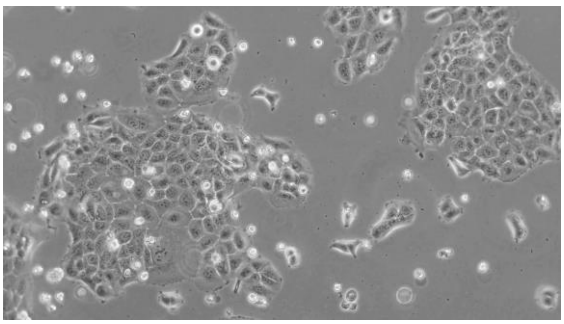
Cell Designation:	KeratinoSens®	Source:	Givaudan
Cat-N°:	RE232	Lot-N°:	92-191122JP03
Packaging Unit:	2.5 million cells / vial	Assay Medium:	H
Passage:	<25	Storage:	below -150°C (e.g. liN_2)
Approval Date:	12.12.2019	Approved by:	
Expiry Date:	22.11.2021		

Susan Ciura (Head of Quality Control)

QUALITY CONTROL

	Batch Results	Specification Limits
Cell Count	2.36E+06	≥ 90 % of nominal cell count
Homogeneity (cell count)	97 %	≤ 90 %
Viability (after thawing)	97 %	≥ 90 %
Aggregation	1.2	≤ 2.0
Debris Ratio	0.2	≤ 1.0
Proliferative Capacity	100 %	≥ 70 %
Sterility (bacteria, yeast, fungi)	passed	sterile after 4 days
Sterility (mycoplasma)	passed	negative by PCR
Morphology	passed	unaltered to reference
$EC_{1.5}$ DNCB	2.07 µM	≤12.5 µM
$EC_{1.5}$ EGDMA	45.08 µM	30 - 100 µM
$EC_{1.5}$ Lactic Acid	> 2000 µM	≥ 1000 µM

MORPHOLOGY:



Morphology of KeratinoSens Assay Ready Cells

acCELLerate GmbH
Osterfeldstraße 12-14
22529 Hamburg, Germany

managing directors
Dr. Oliver Wehmeier
Dr. Alexander Loa

bank account details
Commerzbank Hamburg AG
IBAN DE06200400000327703500
BIC COBADEHHXXX

company details
VAT ID No. DE294633185
register number: HRB 131393
registered seat: Hamburg, Germany

METHODS:

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. 50 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. Equal aliquots of each cell samples were dispensed into a 96 well-plate. A cellular dye was added to the well to obtain fluorescent signal which is proportional to the viable cell count.

Proliferative Capacity: 1,0E+04 cells from the Assay Ready Cell samples and from a reference of a continuously passaged culture were seed in a 96-well plate (8 replica each). After 48 hours of cultivation the proliferation of the cells was determined by addition of a metabolic cell dye (Resazurin). The proliferative capacity of the Assay Ready Cells is calculated in percent of the exponentially growing reference. (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). The 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 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.