

# CERTIFICATE OF ANALYSIS

## instaCELL TNF- $\alpha$ Potency assay kit

**CatN°: SF320-01**

**Lot#: TP-230210**

**Expiry Date: 17.08.2024**

### PRODUCT DEFINITION

Test kit to assess the potency of TNF- $\alpha$  products by their application to cultures of mammalian cells and the subsequent determination of cell viability.

### QUALITY SPECIFICATION OF THE CELLS

	Batch Quality Control	Specification Limits
Cell Count	<b>3.2E+06</b>	1.00E+06<>5E+06
Homogeneity (cell count)	<b>98%</b>	≥ 90%
Viability (after thawing)	<b>97%</b>	≥ 90%
Proliferative Capacity	<b>94%</b>	≥ 70%
Debris/Cell Ratio	<b>0.2</b>	≤ 1.0
Aggregation	<b>1.2</b>	≤ 2.0
Sterility (bacteria, yeast, fungi)	<b>passed</b>	negative after 7 days
Sterility (mycoplasma)	<b>passed</b>	negative by PCR
Morphology	<b>passed</b>	unaltered to reference
EC <sub>50</sub> TNF- $\alpha$ Ref.	<b>20.9 pg/ml</b>	6 pg/ml – 21 pg/ml

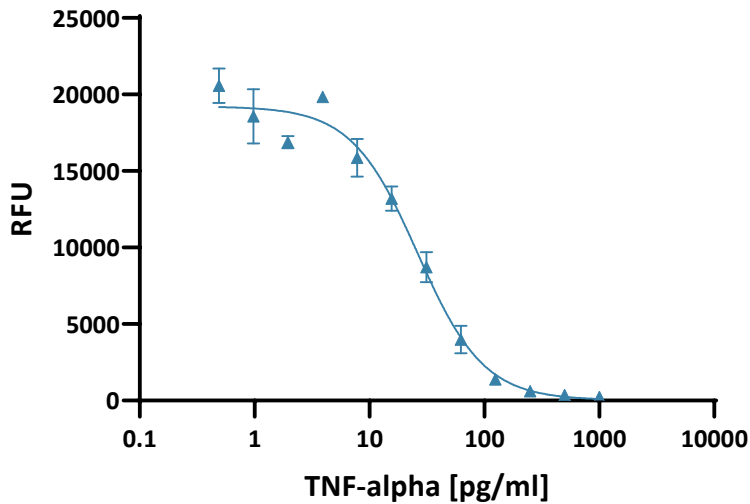
### KIT CONTENT

	Cat-N°	Lot#	Storage	Quantity
Recovery Buffer A	MD163-01	91-220822MD01	-20°C	1
Assay Buffer A	MD363-06	91-220822MD02	-20°C	1
TNF- $\alpha$ Stock Solution	RX517-01	91-220817NR01	-80°C	1
Actinomycin D	KR115-08	220613	RT	1
Resazurin	RX718-01	91-230201ML02	-20°C	1
Assay Ready L-929 Cells	RE772K	92-221114ML01	< -140°C	1
96-well Assay Plate	ZG14-08	0023821	RT	1

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.



## POTENCY ASSAY



Dose-Response curve of the international TNF- $\alpha$  Standard according to the assay protocol.

## METHODS

**Cell Viability Parameters** (cell count, viability, aggregation, amount of debris) are determined in a CASY TT automatic cell counter. Homogeneity is analyzed in a plate-based assay.

**Proliferative Capacity** compares the mean growth rates of freshly thawed cells in relation to exponentially growing cells over 72 hours.

**Sterility** is tested by inoculation of aerob and anaerob growth broths (Tryptic Soy and Thioglycollate for bacteria, yeast and fungi) with samples and cultivation over a course of 7 days.

**Mycoplasma** are detected by PCR using a mycoplasma detection kit.

**Species Identity** is tested by amplification of a specific fragment of 18S rRNA coding region via multiplex PCR (dog, mouse, Chinese hamster, human, monkey, rat, pig and bovine).

**Human Cell Identity** is performed by STR analysis (DNA fingerprinting). Markers: D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX and vWA, DYS391, D2S441, D1S1656, D2S1338, Y indel, D12S391, D19S433, D22S1045, D10S1248, SE33, Amelogenin.

**Potency Assay:** The assay was performed according to the instaCELL protocol.

**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.

