

## Using L-929 Assay Ready Cells to measure the Potency of TNF- $\alpha$ Neutralizing Biotherapeutics

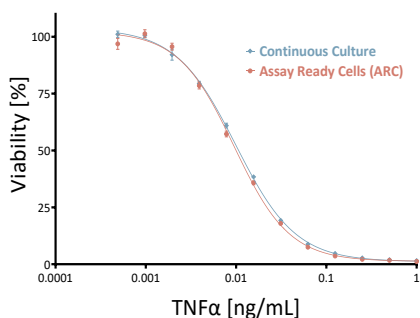
Therapeutic antibodies and soluble proteins which neutralize the activity of TNF- $\alpha$  are used to regulate overshooting inflammatory responses. To control the potency of the biotherapeutic drugs murine fibroblast L-929, to which TNF- $\alpha$  is cytotoxic (Humphrey + Wilson, 1999), are used. Neutralizing antibodies can rescue L-929 cells from the cytotoxic effect.

We here demonstrate, that cryopreserved L-929 cells can be used in a TNF- $\alpha$  neutralization assay like a reagent without prior cultivation. The prequalified assay ready cells (ARCs) are at least as precise as cells from a continuous culture. And variances in cell handling or passaging are widely eliminated from the assay.

### prerequisites

*L-929 Assay Ready Cells are as sensitive to TNF- $\alpha$  as cultured cells*

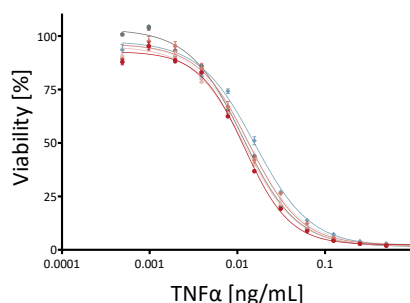
L-929 cells from a continuously passaged culture and ARCs which have been seeded into the assay plates directly after thawing are killed by cytotoxic TNF- $\alpha$  at the same dose at an EC50 of 0.0099 ng/ml versus 0.0095 ng/ml, respective (Fig.1).



**Fig.1:** Continuously cultured L-929 and ARCs perform comparably

The cytotoxic potency of a given TNF- $\alpha$  preparation can be precisely determined with L-929 ARCs. In six independent experiments performed at different days

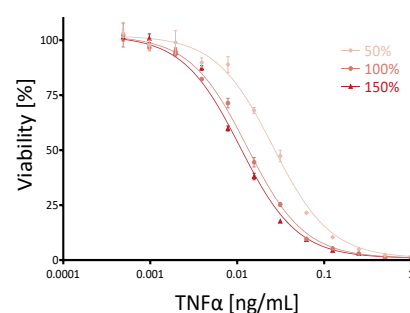
using six different aliquots of ARCs an average EC50 of 0.013 ng/ml was determined at a CV of 12% (R2=0.997) and an



**Fig.2:** TNF- $\alpha$  is cytotoxic to L-929 ARCs

average hill slope of 1.466 (Fig.2).

The accuracy of ARCs was confirmed in a spiking experiment where nominal con-



**Fig.3:** Dilutional Linearity of TNF- $\alpha$

centrations of 50%, 100% and 150% of TNF- $\alpha$  were applied and potencies of 49%, 100% and 141% were determined, which is an average accuracy of 95.5 (Fig.3).

### method (TNF- $\alpha$ Potency Assay)

L-929 cells from continuous culture or from assay ready stock were seeded into a 96 well plate at a density of 15.000 cells/well and let adhere overnight. A serial dilution of TNF- $\alpha$  (Gibco #PHC3015) plus 1 $\mu$ g/ml Actinomycin D was added to the cells and incubated for 24h. The relative viability of the cells was determined by the addition of a metabolic dye (Resazurin) in a fluorescence read-out.

**The potency of TNF- $\alpha$  neutralizing biotherapeutics can be precisely determined on L-929 assay ready cells.**

The potency of three TNF- $\alpha$  neutralizing biotherapeutics, two antibodies, Infliximab and Adalimumab, and a soluble TNF- $\alpha$  receptor fusion protein, Etanercept, was determined on L-929 assay ready cells.

Five to six independent experiments were performed, with individual aliquots of assay ready cells, on different days, displaying precision for the IC50 value. (Fig.4-6)

The CV of the IC50 value is 16% for Adalimumab (Fig. 4), 9% for Etanercept (Fig.5), and 9% for Infliximab (Fig.6). The nominal potencies of drugs are confirmed within the accepted confidence interval of 0.95 (80% <> 125%): 91% to

EUROPEAN OFFICE

+49 (160) 987 577 56

acCELLerate GmbH  
Osterfeldstraße 12-14  
22529 Hamburg - Germany

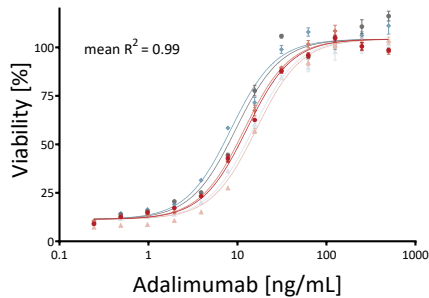
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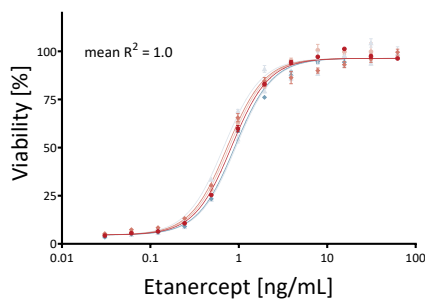
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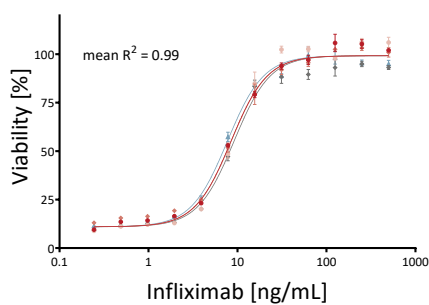
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400 Route 518  
Skillman, NJ 08558 - USA



	Mean	STD	CV
Hill slope	1.76	0.21	12%
<b>IC 50</b>	<b>12.15</b>	<b>1.98</b>	<b>16%</b>
Top RFU	19096	2227	12%
Bottom RFU	2029	328	16%



	Mean	STD	CV
Hill slope	2.11	0.08	4%
<b>IC 50</b>	<b>0.79</b>	<b>0.07</b>	<b>9%</b>
Top RFU	19533	3819	20%
Bottom RFU	952	133	14%



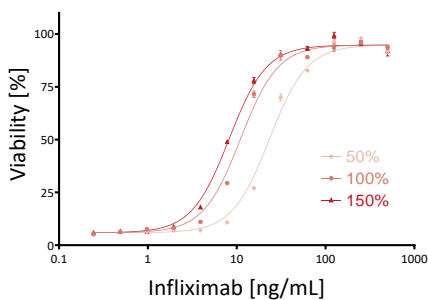
	Mean	STD	CV
Hill slope	2.35	0.44	19%
<b>IC 50</b>	<b>8.65</b>	<b>0.76</b>	<b>9%</b>
Top RFU	18494	1978	11%
Bottom RFU	2237	452	20%

**Fig. 4-6:** Inhibition of TNF- $\alpha$  using Adalimumab, Etanercept and Infliximab

110% for Adalimumab, 89% to 113% for Etanercept and 93% to 107% for Infliximab.

applied and potencies of 43.6%, 100% and 145.2% were determined, which is an average accuracy of 92.0 %.

metabolic dye (Resazurin).



**Fig. 7:** Dilutional linearity of Infliximab

The accuracy of the neutralization assay was tested with Infliximab (Fig.7). Nominal doses of 50%, 100% and 150% were

#### method (TNF- $\alpha$ Neutralization Assay)

L-929 cells from assay ready stock were seeded into a 96 well plate at a density of 15.000 cells/well and let adhere overnight. A serial dilution of the respective neutralizing biotherapeutic was preincubated with TNF- $\alpha$  (Gibco # PHC3015) at a concentration of 0,158 ng/ml (EC90) for 1h at RT. Then the neutralized dilutions were applied to the cells together with Actinomycin D (1 $\mu$ g/ml). After incubation for 24h, the relative viability of the cells was determined by the addition of a

#### conclusion

L-929 assay ready cells are a convenient and flexible tool to precisely determine the bioactivity of TNF- $\alpha$  and the potency of TNF- $\alpha$  neutralizing biotherapeutics.

#### related products

<a href="#">Assay Ready L929</a>	RE772k
<a href="#">TNF-<math>\alpha</math> neutralization kit</a>	SF310-01
<a href="#">TNF-<math>\alpha</math> potency kit</a>	SF320-01

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ALWAYS PERFORMING !

