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NEUTRAKINE™ IL-6 ANTIBODY (69001-1-IG)

Protocol for datasheet validation experiment

The bioassay for the IL-6 (HZ-1019) is performed as a measure of stimulation of proliferation of a mouse hybridoma cell line (Proteintech, anti-GST Clone 3G12B10). Pre-incubation with neutralizing antibody (69001-1-Ig) inhibits IL-6-induced proliferation of hybridoma cells as measured by Promega CellTiter 96® AQueous One Solution Reagent (G35812).

1. Reconstitute antibody according to datasheet and prepare a 4x starting concentration of 69001-1-Ig (see table below). Following the Standard Plate Map, perform serial dilution of NeutraKine antibody by diluting 2-3-folds in the 96 well assay plate from columns 3-12 in assay media for a final volume of 50 µL. Columns 1 and 2 are to be used as cell only and cytokine only controls, respectively.
2. Add 100 µL of the assay media to column 1 and add 50 µL of the indicated concentration of the cytokine (see table below) to columns 2-12 in assay media. Add an additional 50 µL of assay media to column 2.
3. Incubate the plate at 37°C for approximately 2 hours in 5% CO_2 humidified incubator.
4. At the end of two hours prepare cell suspension of hybridoma cells at $2x10^5$ cells/mL and add 100 µL of prepared cell suspension to wells 1-12.
5. Incubate the cells for 72 hours at 37°C in 5% CO_2 humidified incubator.
6. Following completion of the 72 hours incubation assay the cell viability CellTiter 96® AQueous One Solution Reagent (G3581).
7. Graph the activity using analytical/ statistical software (such as GraphPad Prism) to obtain 50% neutralization dose (ND50).

Standard Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cell Only Control	Cytokine Only Control	Neutrakine Antibody+ Cytokine									
B			Neutrakine Antibody+ Cytokine									
C			Neutrakine Antibody+ Cytokine									
D			Neutrakine Antibody+ Cytokine									
E			Neutrakine Antibody+ Cytokine									
F			Neutrakine Antibody+ Cytokine									
G			External Standard for Comparison - Control									
H			External Standard for Comparison - Control									

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NeutraKine and cytokine recommended concentrations

Target	NeutraKine Cat No.	4X Starting Concentration (ng/mL)	Recommended Cytokine Concentration (ng/mL)
IL-6	69001-1-Ig	200	1
IL-3	69004-1-Ig	1000	0.5
IFN gamma	69007-1-Ig	5000	0.4
IFN alpha 2A	69008-1-Ig	10000	100
TNF alpha	69002-1-Ig	100	5
GM-CSF	69003-1-Ig	200	0.2
IL-23 P40	69006-1-Ig	1000	4
IL-4	69005-1-Ig	10,000	0.4
Beta NGF	69009-1-Ig	250	2