

Validation of a flow cytometry based whole blood Natural Killer (NK) cell cytotoxicity assay as Immunity marker

Background & Introduction:

Natural Killer (NK) Cells are lymphocytes, best known for killing virus infected cells and malignant cells. Most normal healthy cells express MHC I receptors which mark the normal healthy cells as self. Inhibitory receptors on the surface of the NK cell recognise cognate MHC I and this switches off the activity of NK cell, preventing it from killing normal cells. Cancer cells and infected cells often lose their MHC I, leaving them vulnerable to NK cell cytotoxicity. NK cell releases cytotoxic granules which lead to lysis of the target cells. NK cell cytotoxicity is widely used as primary indicator for immune function. NK cells not only works as first line defence against pathogenic invasion but also serves as immune surveillance for malignant proliferations.

Method Summary:

An accurate NK cell activity assay was optimized and validated to serve as Immunity marker. The assay involved isolation of NK cells from whole blood using PBMC extraction, stimulation of isolated NK cells and incubation of un-stimulated and stimulated NK cells with cryopreserved K562 cells pre-stained with green fluorescent membrane dye. K562 cells lack MHC- I & II markers which makes it target for NK Cells. After incubation of target and effector cells, a red fluorescent DNA dye was added to label the target cells permeabilized by NK Cell activity. The live and dead cells were determined in flow cytometry and the NK cell activity is calculated using the response from un-stimulated and stimulated NK cells.

A fit for validation was conducted by evaluating Intra & Inter assay precision, repeatability, specificity, stability, and robustness prior to study sample analysis.

Isolate effector cells from individual blood samples using Ficoll-Hypaque procedure

Thaw cryopreserved K562 cells labelled with lipophilic green fluorescent membrane dye

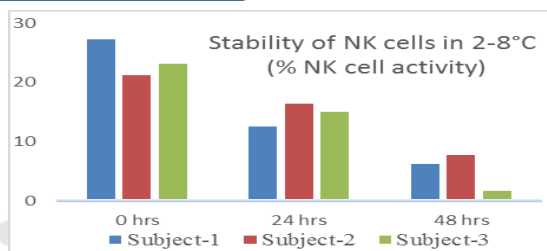
Prepare reaction mixture with fixed number of NK cells in complete media

Stimulate of K562 cells using recombinant interleukin-2 (IL2)

Mix NK cells (unstimulated and stimulated) K562 cells

Add DNA staining solution and measure fluorescence using FACS

Result & analysis



Robustness: Incubation (5% CO₂, 37°C) duration

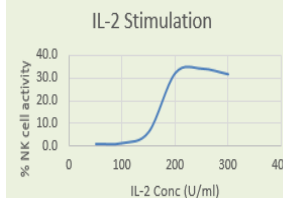
IL-2 Stimulation	K562 & NK cells	% NK Cell activity
60 mintues	180 minutes	37.37
90 mintues	180 minutes	31.86
60 mintues	240 minutes	27.70

Intra & Inter Precision (% NK cell activity)

Intra Assay Precision		Individual 1	Individual 2
Day 1 (N=6)			
Analyst-1	Mean	34.0	13.2
	CV	7.9	13.0
Analyst-2	Mean	38.9	17.2
	CV	12.1	17.3
Day 2 (N=6)			
Analyst-1	Mean	24.7	14.7
	CV	12.5	10.6
Analyst-2	Mean	33.1	13.8
	CV	10.7	17.1
Inter Assay Precision		18.8	17.3

Specificity (200 U/ml)

Stimulating agent	% NK cell activity
IL-2	32
IL-4	1
IL-6	1
IL-8	1
IL-10	2



Conclusion:

The method is validated as precise, robust, reproducible, specific and ready to perform study sample analysis.

The method is capable to accurately detect the in vitro NK cell activity and act as suitable Immunity marker to monitor the correlation between NK cell activity and immunity status.

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