

# NK CELL

Natural Killer Cell  
Research Solution

Providing Innovative Reagents for Life Sciences since 2006





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Distributed by:

**CliniSciences Group**



# NK

Natural Killer (NK) cells are an important member of the human immune cell family, primarily distributed in peripheral blood, liver, and spleen. NK cells possess four major immune defense functions: immune defense, immune regulation, immune surveillance, and immune homeostasis. NK cells can eliminate infected cells, prevent viral spread, and promptly recognize and eliminate cells undergoing malignant transformation. Additionally, NK cells can activate new healthy cells, accelerate metabolism and circulation in the body, aid in restoring bodily functions, delay aging, and prevent diseases. Due to their allogeneic use and innate anti-tumor capabilities, NK cells have significant advantages in cell therapy and are one of the key research areas in the field.



# NK Cell Separation

The sources of NK cells mainly include peripheral blood, umbilical cord blood, NK cell lines (such as NK-92), and induced pluripotent stem cells (iPSCs). Currently, allogeneic peripheral blood mononuclear cell (PBMC) isolation is the mainstream method for obtaining NK cells in clinical settings. The commonly used method in the industry involves initially isolating PBMCs, followed by enrichment and expansion of NK cells through activation using feeder cells or pure factors. TransGen Biotech offers a ready-to-use peripheral blood lymphocyte separation solution with high separation efficiency, which has been classified as a Class I medical device by the National Medical Products Administration (NMPA), fully meeting customer needs.

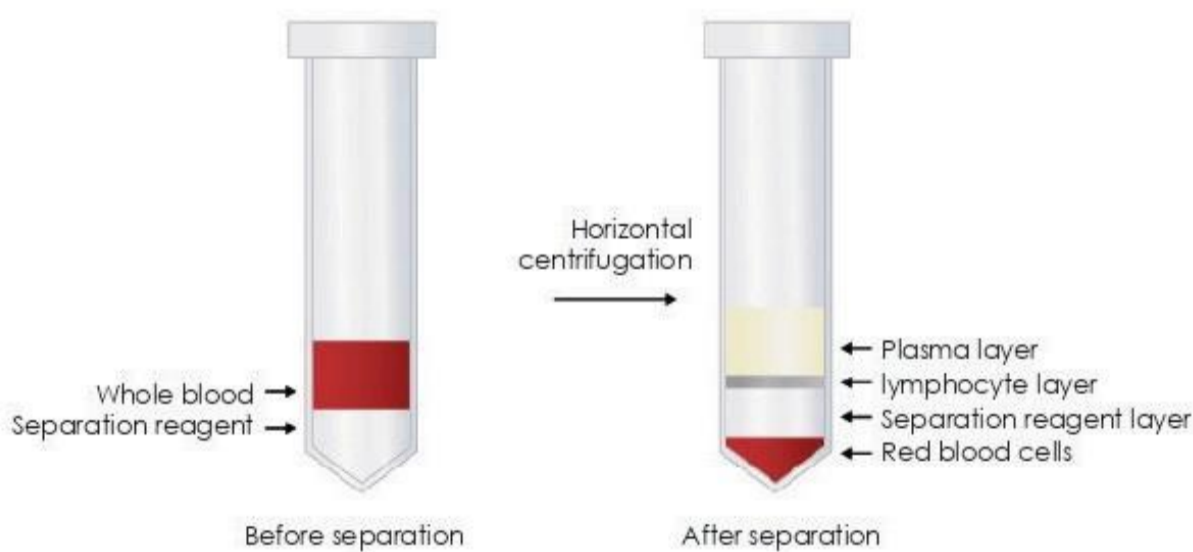
## Human Peripheral Blood Lymphocyte Separation Solution (FB102)

### Feature

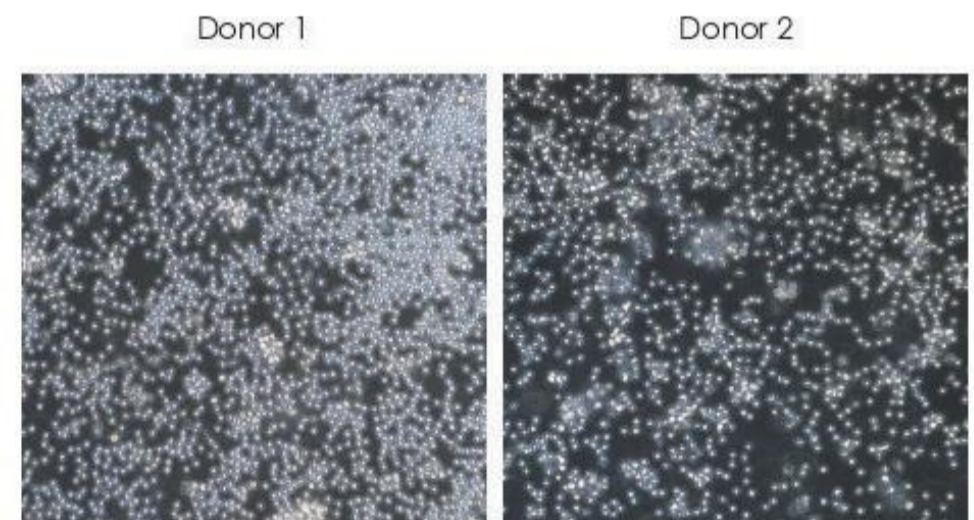
- The osmotic molarity of this solution is similar to that of human peripheral blood cells.
- The quantity of separated lymphocytes is greater than  $1 \times 10^6$  cells/mL whole blood.
- The post-separation viability of lymphocytes > 95%.
- This product is ready-to-use, and the lymphocytes separated under sterile conditions can be used for in vitro culture and immunological assays.

### Experimental data

#### Separation diagram



#### The cell morphology is well preserved



The yield of cells is greater than  $1 \times 10^6$  cells/mL whole blood, with a viability of over 95%.

Testing Parameters	Donor 1	Donor 2	Donor3	Donor 4	Donor 5
Number of cells harvested per mL of whole blood	$1.51 \times 10^6$	$1.78 \times 10^6$	$1.12 \times 10^6$	$1.33 \times 10^6$	$1.5 \times 10^6$
lymphocyte viability	98.22%	98.59%	97.08%	98.41%	99.28%



# NK cell amplification

The key factor for utilizing natural killer (NK) cells in cell therapy is the availability of large quantities of highly pure and active NK cells. When expanding NK cells in vitro, the use of cytokines can effectively expand NK cells and enhance their activity. TransGen Biotech offers a serum-free, xeno-free pure factor-based in vitro induction and expansion culture reagent specifically designed for NK cell culture. It exhibits excellent performance and enables the in vitro expansion of large quantities of highly pure NK cells.

## ArtMedia<sup>®</sup> Human NK Cell Serum-Free Expansion Kit (MK101)

### Feature

- **Safe and Stable**

No exogenous components, clear chemical composition.

No serum, only a small amount of inactivated autologous plasma required.

Pure factor reagent kit, no need to use feeder cell layers.

Contains phenol red, free of antibiotics.

- **Strong amplification capability**

100-200 fold expansion in 14 days

200-400 fold expansion in 21 days

- **High purity of NK cells**

14-day purity of CD3-CD56+  $\geq$  90%, CD3-CD16+  $\geq$  85%, CD56+CD16+  $\geq$  85%

21-day purity of CD3-CD56+  $\geq$  94%, CD3-CD16+  $\geq$  90%, CD56+CD16+  $\geq$  90%

- **Strong cytotoxicity of NK cells**

High cytotoxicity against tumor cells

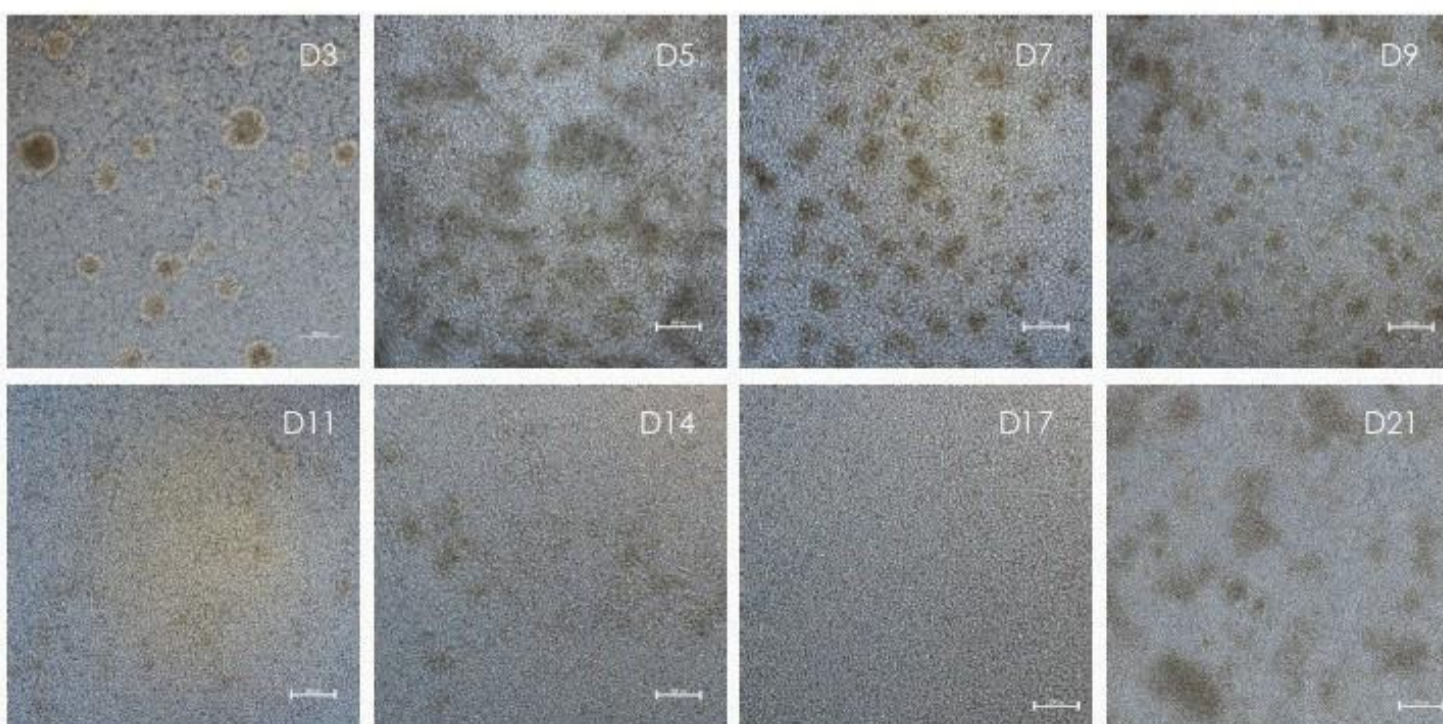
- **Convenient operation, high cost-effectiveness, suitable for industrial production**

Produced and managed according to GMP standards.

### Experimental data

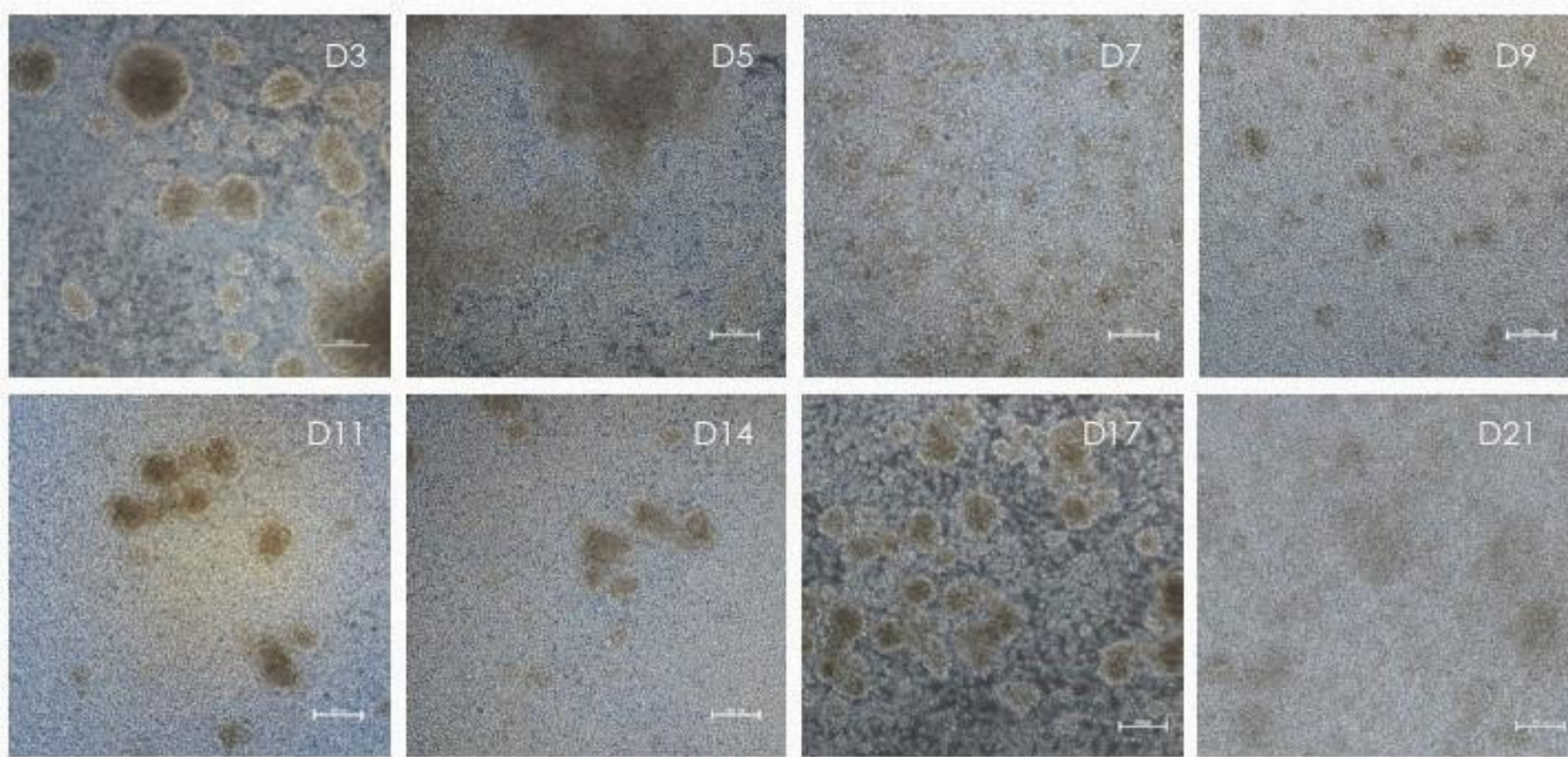
#### Good cell morphology

NK cells activated and expanded after peripheral blood mononuclear cells (PBMCs) separation.

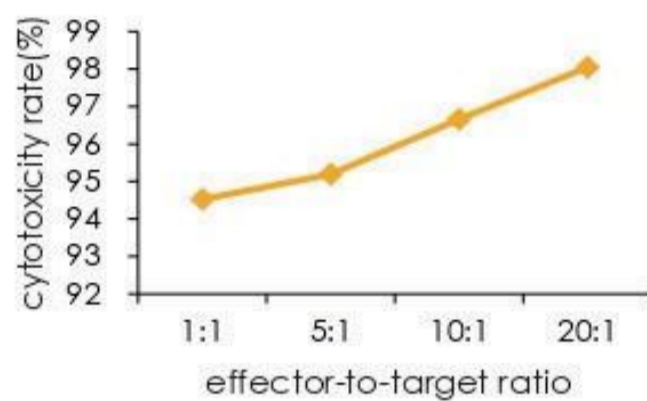




Activated and expanded NK cells sourced from PBMCs obtained via single leukapheresis.

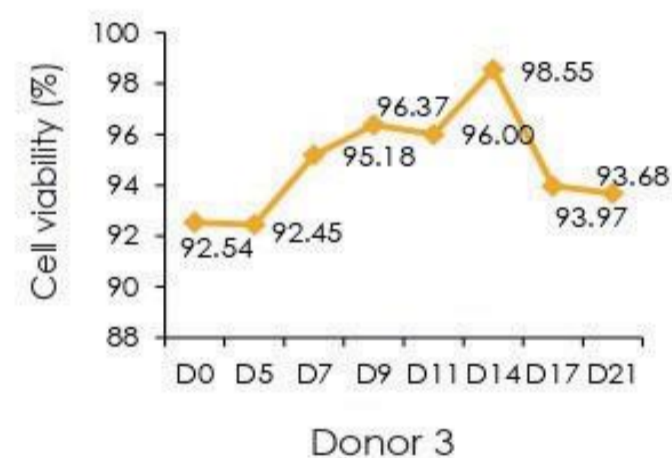
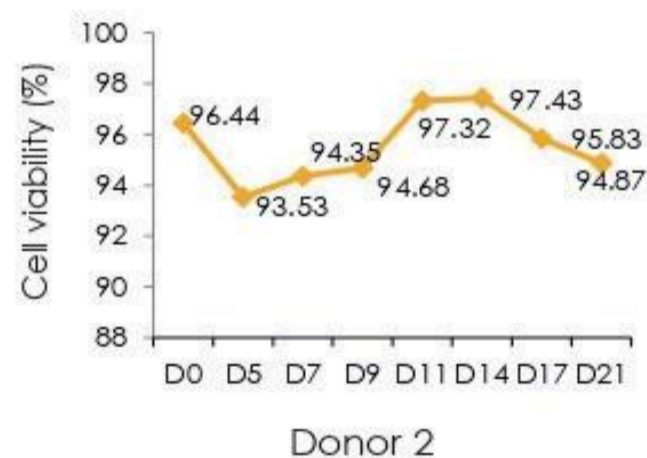
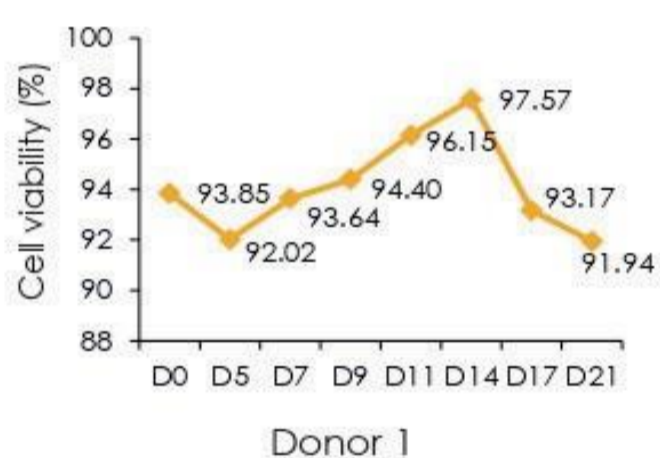


Strong killing effect with high cytotoxicity.

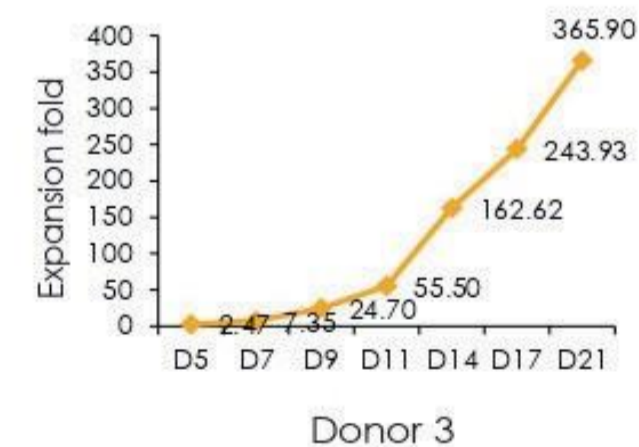
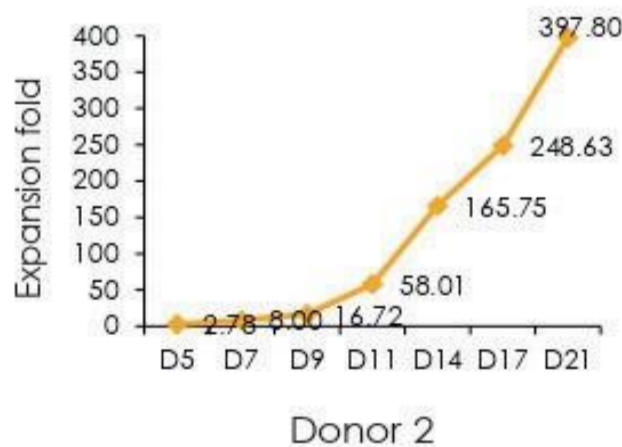
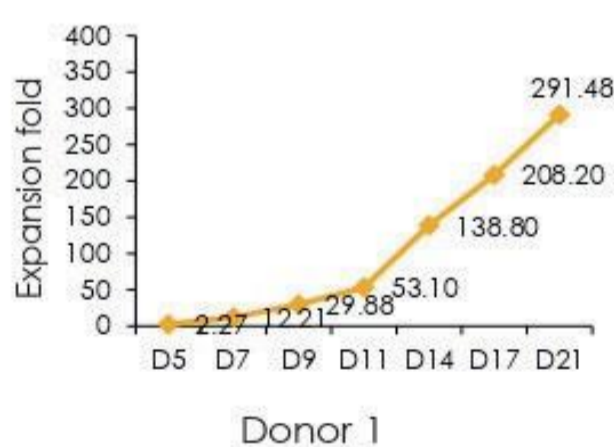


Expanded NK cells sourced from cryopreserved peripheral blood mononuclear cells (PBMCs) from different donors.

Cell viability > 90%.



Strong proliferation capability, with 100-200 fold expansion in 14 days and 200-400 fold expansion in 21 days.

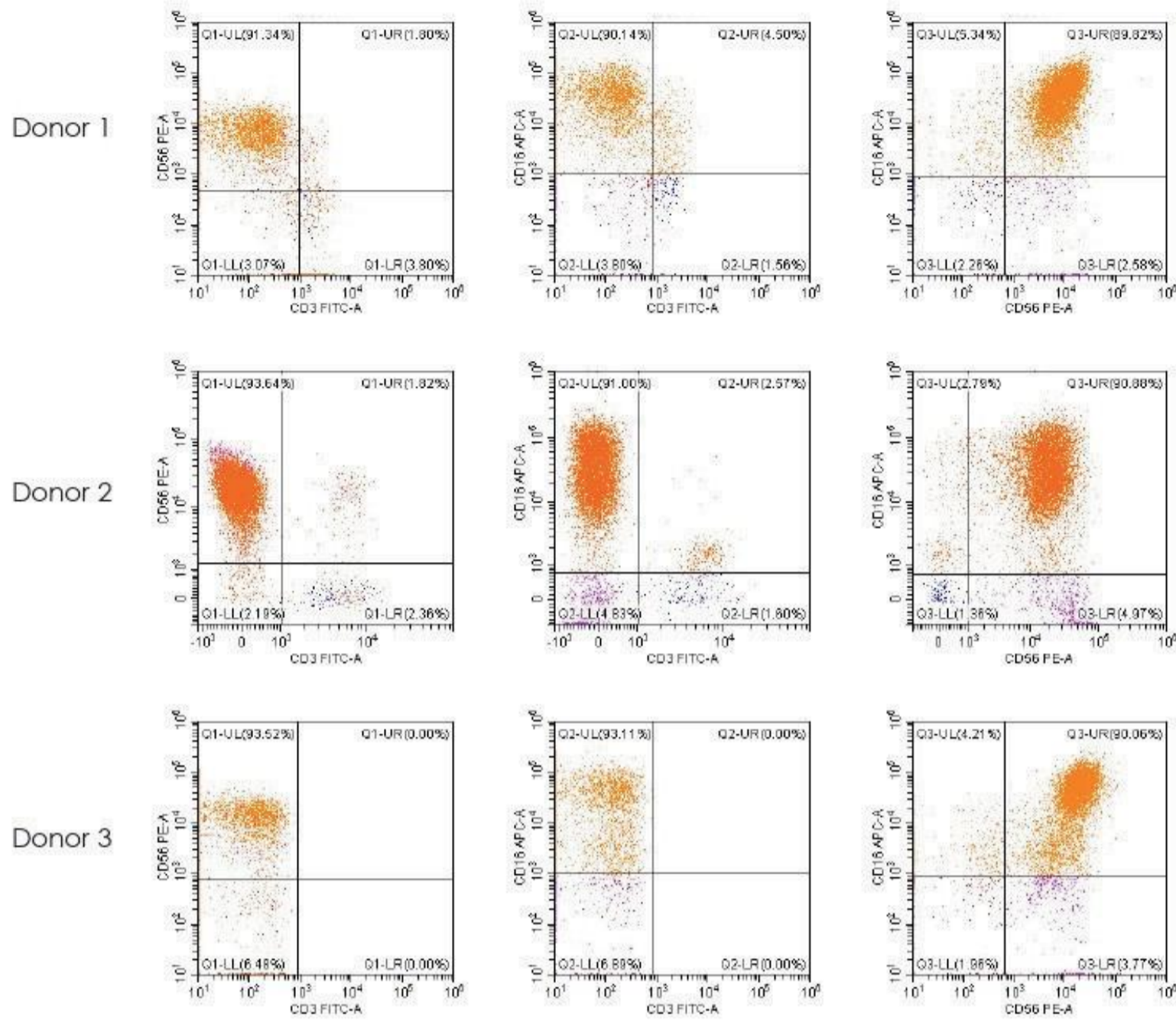


Donor	Donor 1	Donor 2	Donor 3
Initial cell count	$2 \times 10^7$		
Harvested cell count (billion) in 14 days	2.776	3.315	3.252
Harvested cell count (billion) in 21 days	5.83	7.956	7.318

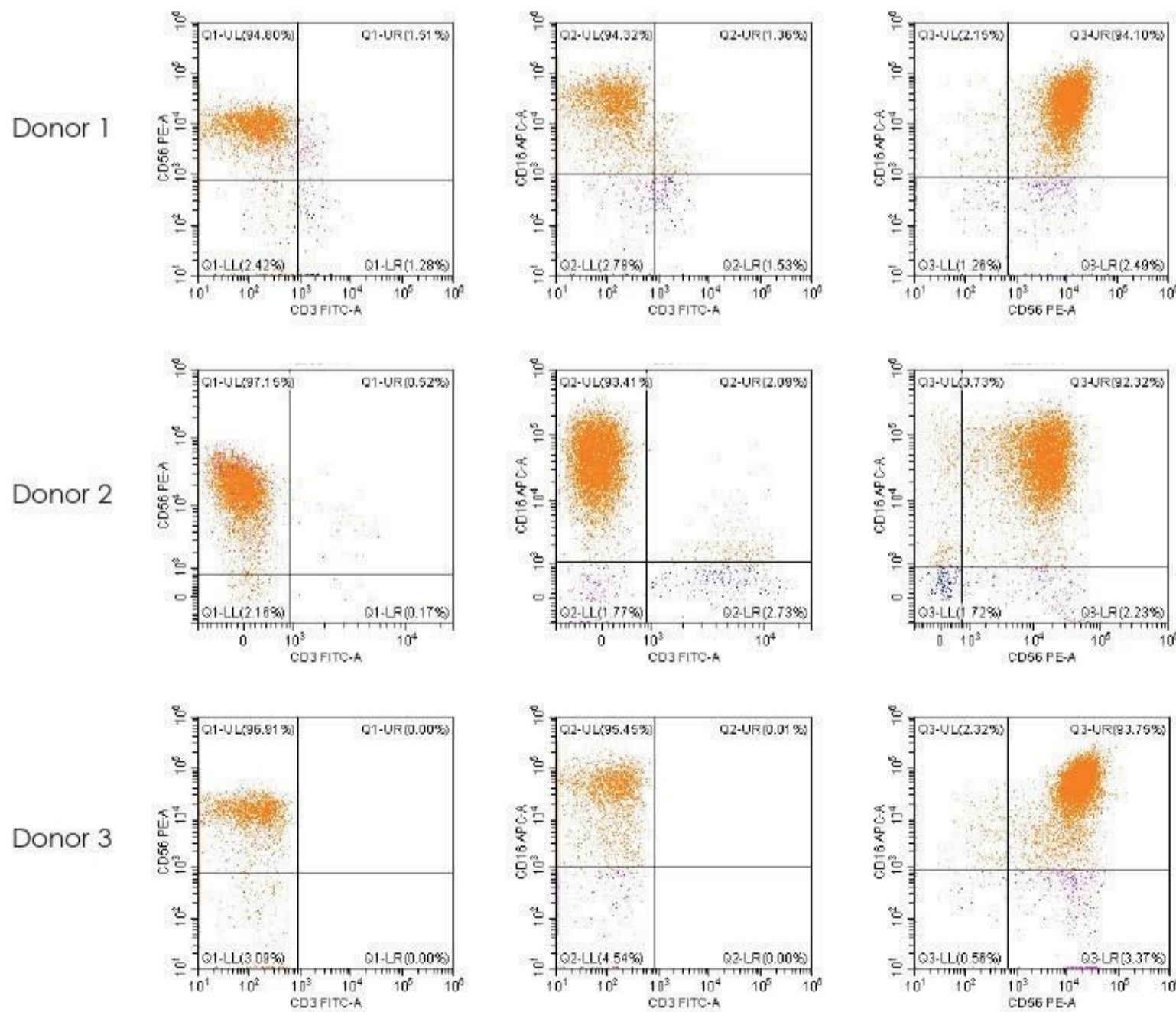


## High cell purity

14-day CD3-CD56+purity  $\geq 90\%$ , CD3-CD16+purity  $\geq 85\%$ , CD56+CD16+purity  $\geq 85\%$



21-day CD3-CD56+purity  $\geq 94\%$ , CD3-CD16+purity  $\geq 90\%$ , CD56+CD16+purity  $\geq 90\%$





# NK cell cryo-preservation

Cells are in a dormant state during ultra-low temperature cryopreservation, during which enzymes in the cells are inhibited, preventing ionization damage, and allowing for long-term storage. Cryopreserved cells can maintain vitality and undergo proliferation after revival. Cryopreservation solution is a crucial product ensuring revival efficiency and cell viability. TransGen offers two types of serum-free cryopreservation solutions, requiring no programmed cooling, with revival rates exceeding 90%.

## TransStem<sup>®</sup> Chemically Defined Xeno-free Cell Cryopreservation Medium-Protein Free (MC102)

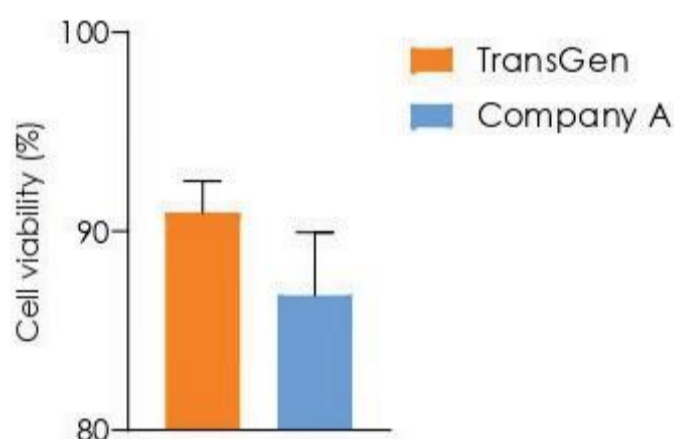
### Product Features

- Safety: Serum-free, xeno-free, protein-free, chemically defined.
- High Efficiency: Significantly enhances cell revival rates, with revival rates exceeding 90% for most cells (including mesenchymal stem cells and immune cells).
- Stability: Does not affect the phenotype and growth status of stem cells, while maintaining their multipotency.
- Convenience: Ready-to-use product, no need for programmed cooling, can be directly transferred to liquid nitrogen for long-term storage after overnight storage at -80°C.
- Reliability: Suitable for the cryopreservation of various cells, including stem cells, immune cells, tumor cells, etc.

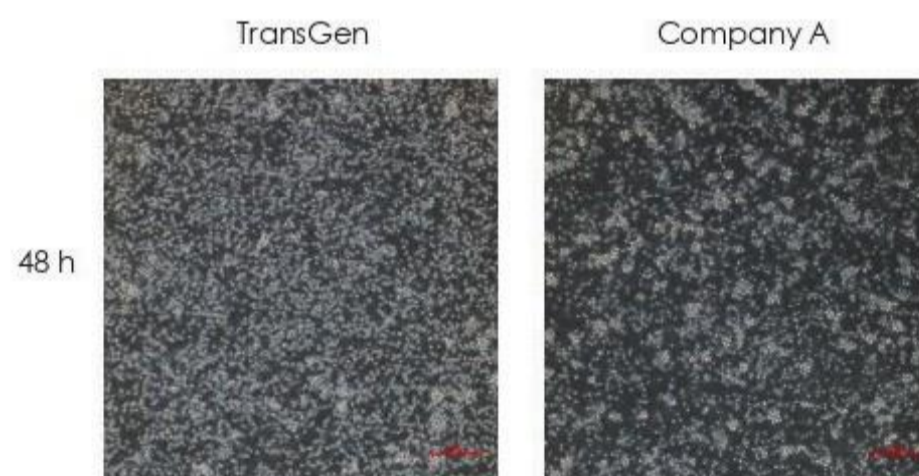
### Experimental data

#### NK cell cryo-preservation

Cell viability > 90%



Good cell morphology



#### Differences from traditional serum-containing cell cryo-preservation solutions

Differences	Traditional serum-containing cell cryo-preservation solution	Serum-free, protein-free cell cryo-preservation solution (containing DMSO)
Serum	Yes	No
Protein	Yes	No
Clear chemical composition	Uncertain	Certain
Cooling program	Require	Not required
Cryo-preservation solution preparation	Use immediately after preparation	Ready to use, store at 4°C
Revival rate	Normal	Outstanding (support high concentration cryopreservation)
Difference between batches	High	Low
Risk of carrying virus	Yes	No
Applicable to clinical research	No, containing animal serum and protein	Yes, clear chemical composition, no animal-derived substances, protein-free



# TransStem<sup>®</sup> Chemically Defined Xeno-free Cell Cryopreservation Medium III-DMSO Free, Protein Free (MC131) (Completely Using Pharmaceutical Grade Materials)

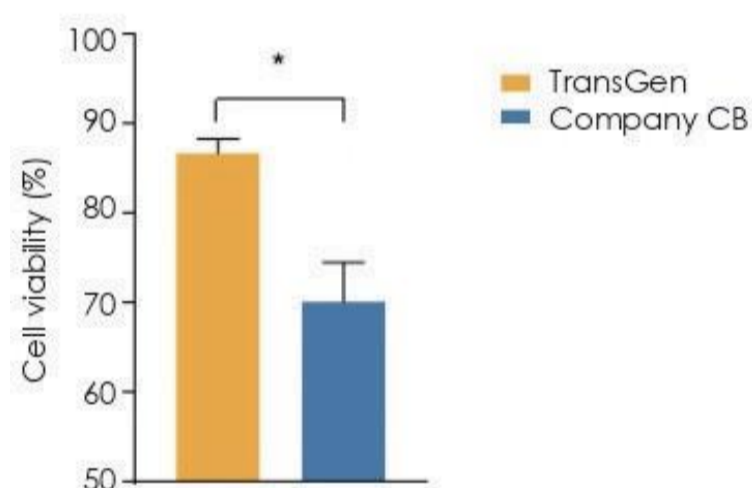
## Product Features

- Safety: Serum-free, xeno-free, protein-free, DMSO-free, clear chemical component, utilizing USP, EP, JP, CHiP graded ingredients, with complete toxicity test.
- High Efficiency: Effectively improves cell recovery viability, with over 90% recovery rate for most cells (including mesenchymal stem cells and immune cells).
- Stability: Does not affect stem cell phenotype and growth status, while maintaining stem cell pluripotency.
- Convenience: Ready-to-use product, no programmed cooling required, can be directly transferred to liquid nitrogen for long-term storage after overnight storage at -80°C.
- Reliability: Suitable for cryopreservation of various cells including stem cells, immune cells, tumor cells, and transformed cell lines.

## Experimental data

### NK cell cryo-preservation

Cell viability >85%



good cell morphology



### Differences from traditional serum-containing cell cryo-preservation solutions

Differences	Traditional serum-containing cell cryo-preservation solution	Serum-free, protein-free, DMSO-free cell cryo-preservation solution
Serum	Yes	No
Protein	Yes	No
DMSO	Yes	No
Clear chemical composition	Uncertain	Certain
Cooling program	Require	Not required
Cryo-preservation solution preparation	Use immediately after preparation	Ready to use, store at 4°C
Revival rate	Normal	Outstanding (support high concentration cryo-preservation)
Difference between batches	High	Extremely low
Risk of carrying virus	Yes	No
Apply for pharmaceutical excipients	Extremely hard	Relatively easy
Applicable to clinical research	No, containing animal serum and protein	Yes, clear chemical composition, no animal-derived substances, protein-free



# NK

## cell quality control

## Mycoplasma detection

"In the considerations for pharmaceutical research and application materials for clinical trial of cell therapy products", it is pointed out that the quality standards for viral and cellular vectors should include mycoplasma, among others. Some species of mycoplasma are pathogenic to humans and animals, posing certain biosafety risks. Therefore, mycoplasma testing is a mandatory requirement stipulated by the biopharmaceutical industry. TransGen Biotech provides two types of mycoplasma detection reagents based on qPCR and luciferase methods, which are simple to operate, highly sensitive, and accurate.

## TransDetect<sup>®</sup> qPCR Mycoplasma Detection Kit (FM321)

### Product Features

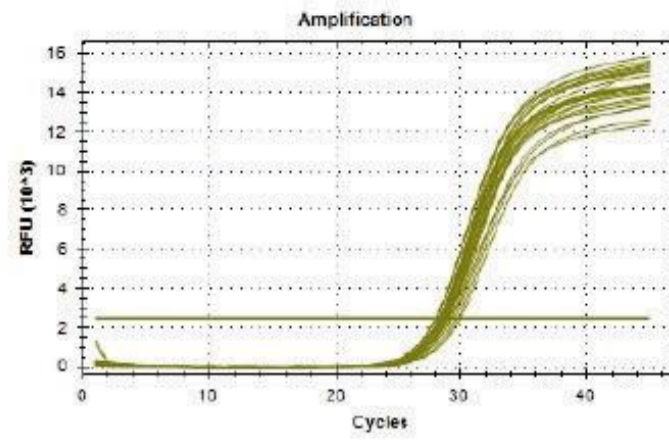
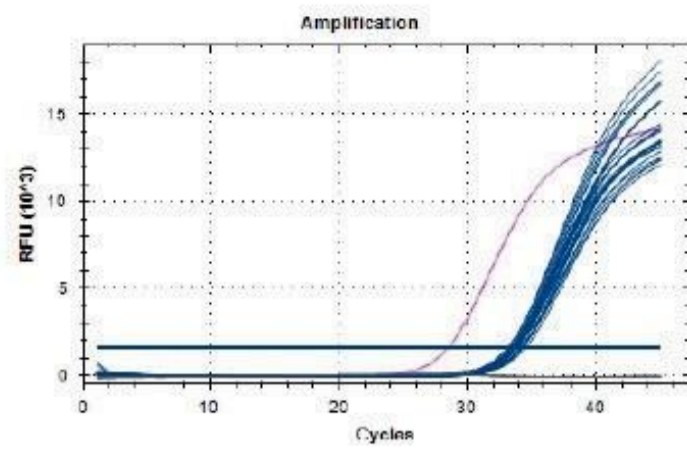
- Compliant with the requirements of nucleic acid amplification technology (NAT) for mycoplasma detection as stipulated in the European Pharmacopoeia (EP 2.6.7) and the Japanese Pharmacopoeia (JP G3).
- Wide Coverage: Validated for the detection of 13 strains of mycoplasma and plasmid DNA, with database comparisons covering at least 129 species of mycoplasma DNA sequences.
- High Sensitivity: Validated mycoplasma strains meet 95% detection requirements, with a detection limit of up to 10 CFU/mL; plasmid standards can reach 1 copy/ $\mu$ L.
- High Specificity: No detection interference from commonly occurring bacteria or production cell genomes, among others.
- dUTP/UDG Contamination Prevention System: Effectively prevents contamination of PCR products, ensuring accurate data.
- High Stability: Product can undergo at least 15 freeze-thaw cycles without significant impact on performance.
- Broad Instrument Compatibility: Compatible with mainstream qPCR instruments, ensuring consistent detection results.

### Experimental Data

High sensitivity, detection limit is 10 CFU/mL

Mycoplasma species	10 CFU/mL detection rate
Mycoplasma gallisepticum	24/24
Mycoplasma synoviae	24/24
Mycoplasma arginini	24/24
Spiroplasma citri	24/24
Mycoplasma fermentans	24/24
Acholeplasma laidlawii	24/24
Mycoplasma hyorhinis	24/24
Mycoplasma orale	24/24
Mycoplasma pneumoniae	24/24
Mycoplasma salivarium	24/24

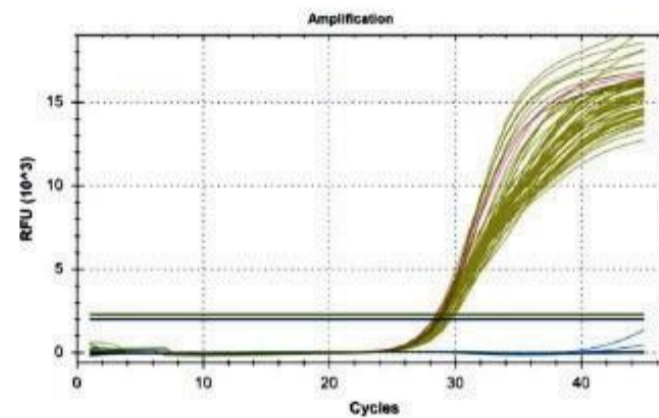




- 10 CFU/mL Fermentation-derived mycoplasma standards
- NTC, NSC (Negative sample extraction product)
- Negative control
- Internal reference

High specificity, no cross-reaction with non-mycoplasma species

Prokaryotic Species	Prokaryotic Species	eukaryotic Species
Lactobacillus acidophilus	Escherichia coli	Pichia pastoris
Streptococcus pneumoniae	mycobacterium smegmatis	Chinese Hamster Ovary
Streptococcus salivarius	Staphylococcus aureus	Sp2/0 call (rat
Enterococcus faecalis	Rhodococcus erythropolis	293T cell
Bacillus subtilis	Micrococcus luteus	Sf9 cell (Spodoptera frugiperda)



- Internal reference signal from all samples
- Mycoplasma signal for each non-mycoplasma species
- Mycoplasma signal for positive controls

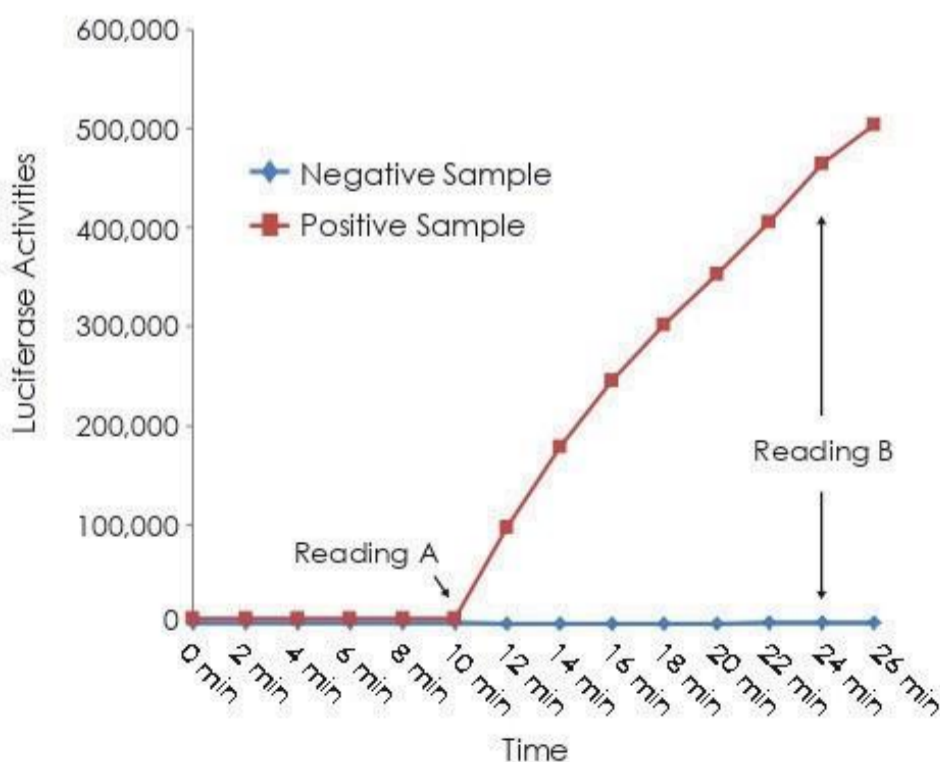
## TransDetect<sup>®</sup> Luciferase Mycoplasma Detection Kit (FM301)

### Product Features

- Detecting mycoplasma that are truly biologically active, with high accuracy.
- High sensitivity, easy operation, time-saving, only takes 25 minutes.

### Experimental Data

High sensitivity, accurate detection of 1:125 diluted original sample



	TransGen	Company L
original sample	+	+
1:5 dilution	+	+
1:25 dilution	+	+
1:125 dilution	+	+/-
1:625 dilution	+/-	-
1:3125 dilution	-	-
negative control	-	-

- + Mycoplasma contaminated sample
- +/- Require retesting for validation
- Sample not contaminated by Mycoplasma



# NK Cell Phenotyping

Obtaining a sufficient quantity of highly pure and active NK cells is crucial for ensuring the effectiveness of cell therapy experiments, and cell phenotyping is an indispensable key step in this process. Currently, flow cytometry is one of the most common methods used to characterize and evaluate NK cell function, which can be achieved by utilizing cell surface markers. TransGen Biotech provides fluorescein conjugated antibodies targeting CD3, CD16, CD56, and CD45 with simple operation, strong specificity, high sensitivity, stable quality, and good repeatability.

**Anti-Human CD3, FITC /PE/APC (HF151/HF152/HF154)**

**Anti-Human CD45, FITC /PerCP-Cy5.5 (HF121/HF125)**

**Anti-Human CD16, FITC/PE/APC (HF191/HF192/HF194)**

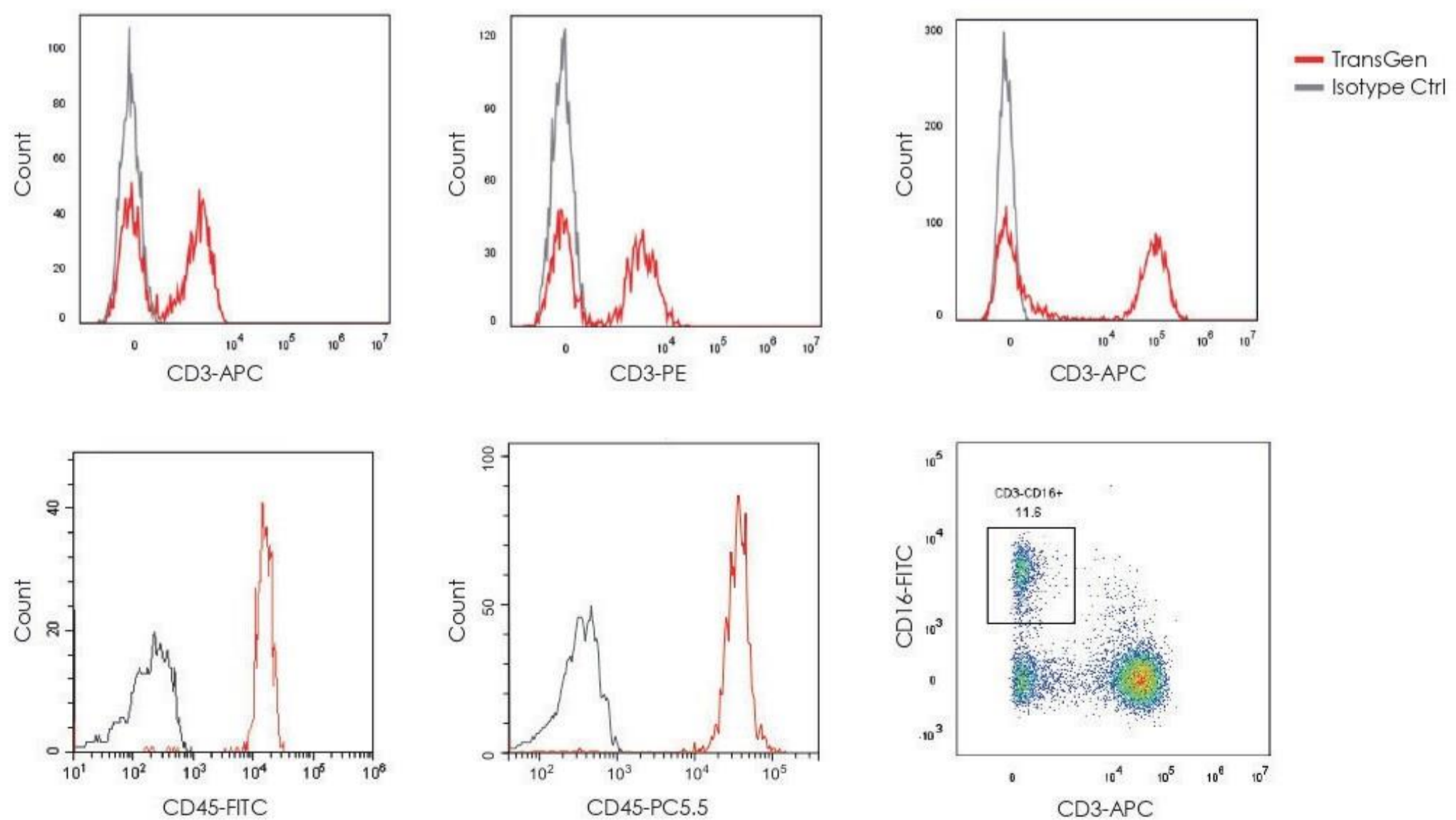
**Anti-Human CD56, PE/APC (HF202/HF204)**

## Product Features

- Monoclonal antibodies with strong specificity and high sensitivity.
- Fluorescein conjugated primary antibodies with no need for self-conjugation, making it simple and time-saving.
- Direct binding to target antigens, unaffected by endogenous antibodies, reducing nonspecific adsorption.
- Available in multiple fluorescein such as APC, PE, FITC, PerCP-Cy5.5, PE-Cy7, APC-Cy7, PerCP, etc.
- Stable quality and good repeatability.

## Experimental Data

### Strong specificity





# NK Cell Activity Assay

The purity of NK cells is an important indicator for assessing the quality and biological activity of NK cells. By measuring the proportion of viable cells within the NK cell population, one can determine the purity of NK cells. TransGen Biotech offers a live/dead cell viability/cytotoxicity assay kit designed for detecting the proportion of live cells within the population.

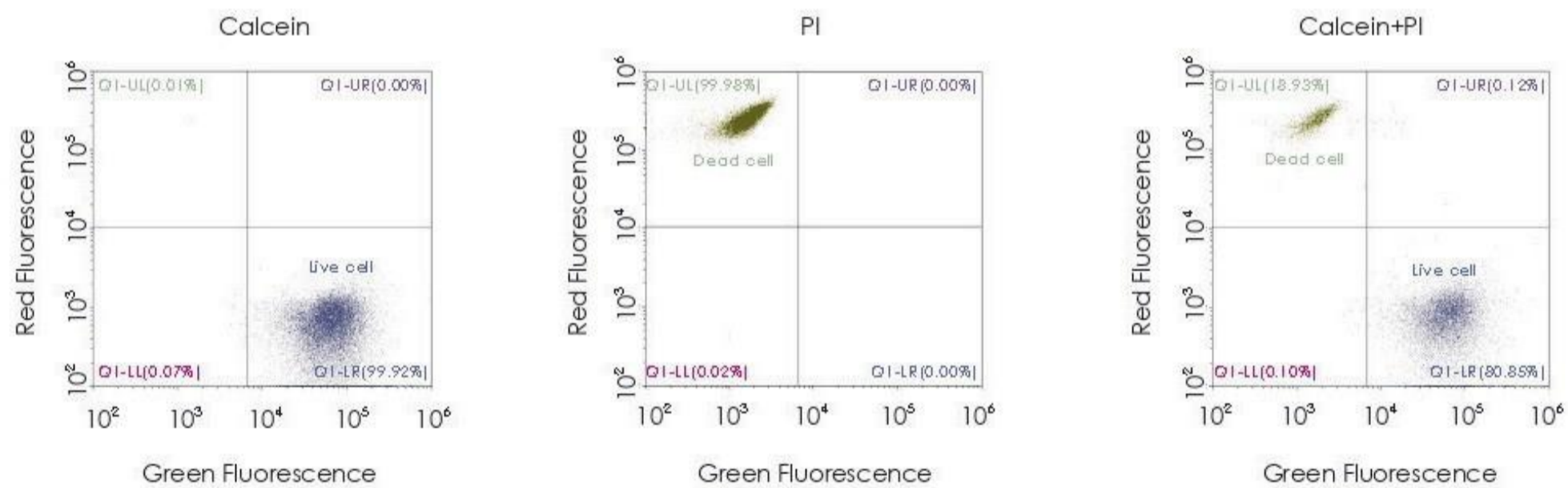
## TransDetect<sup>®</sup> Cell LIVE/DEAD Viability/Cytotoxicity Detection Kit (FC301)

### Product Features

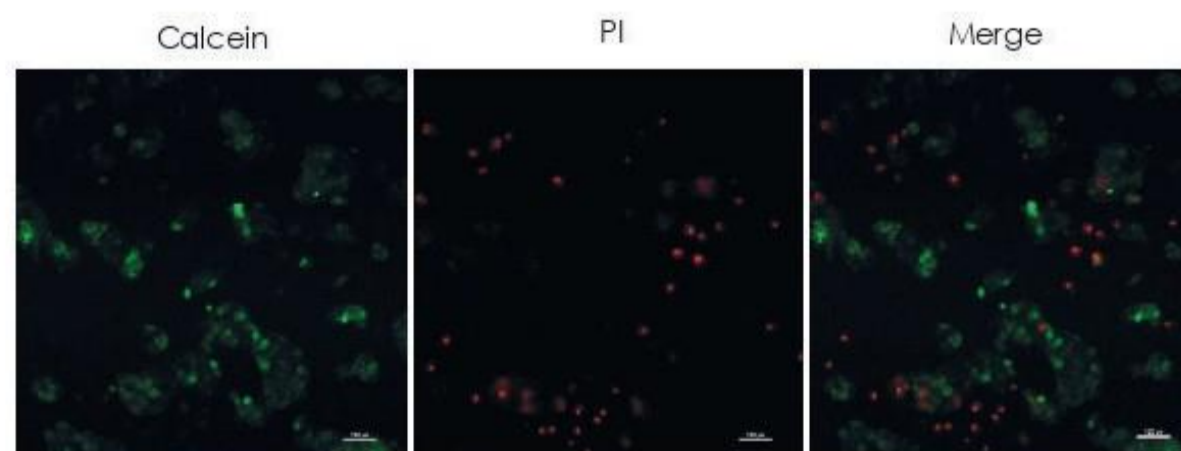
- Simple operation with strong fluorescence specificity.
- Low cytotoxicity.
- Suitable for quantitative analysis or sorting of live and dead cells using flow cytometry.

### Experimental Data

#### Flow cytometry analysis of live and dead cells



#### Fluorescent microscopy for detecting live and dead cells.





# NK Cell Cytotoxicity Assay

NK cells exert their cytotoxic function by directly recognizing target cells and releasing perforin, NK cell cytotoxic factors, TNF- $\alpha/\beta$ , and other factors to induce target cell death. The cytotoxic activity of NK cells can be assessed by co-culturing them with target cells (usually K562 cells) and then measuring the viability of the target cells. TransGen Biotech provides rapid and highly sensitive cell proliferation detection kit and luminescent cell viability kits for NK cell cytotoxicity detection.

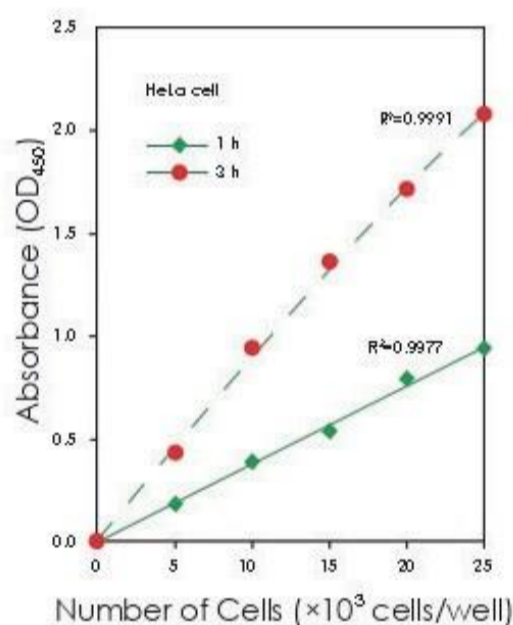
## TransDetect<sup>®</sup> Cell Counting Kit (CCK) (FC101)

### Product Features

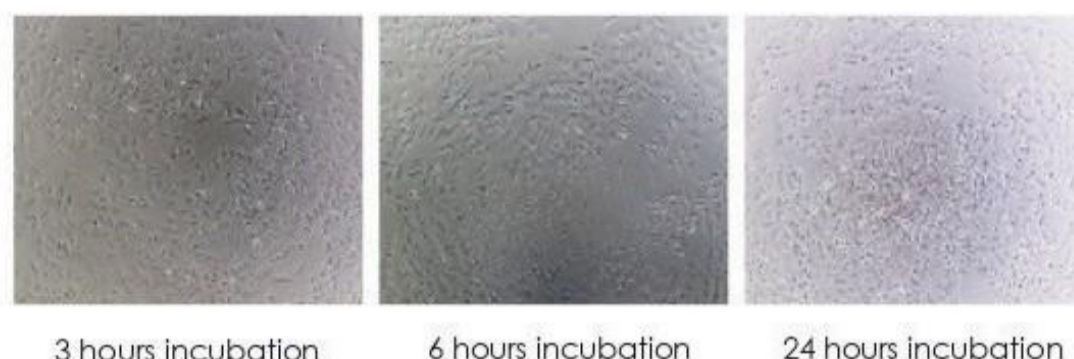
- Fast and sensitive
- Low cytotoxicity
- Wide linear range
- Stable results with good repeatability

### Experimental Data

#### CCK sensitivity test



#### CCK exhibits low cytotoxicity towards cells



3 hours incubation

6 hours incubation

24 hours incubation

## TransDetect<sup>®</sup> Luminescent Cell Viability Detection Kit (FC401)

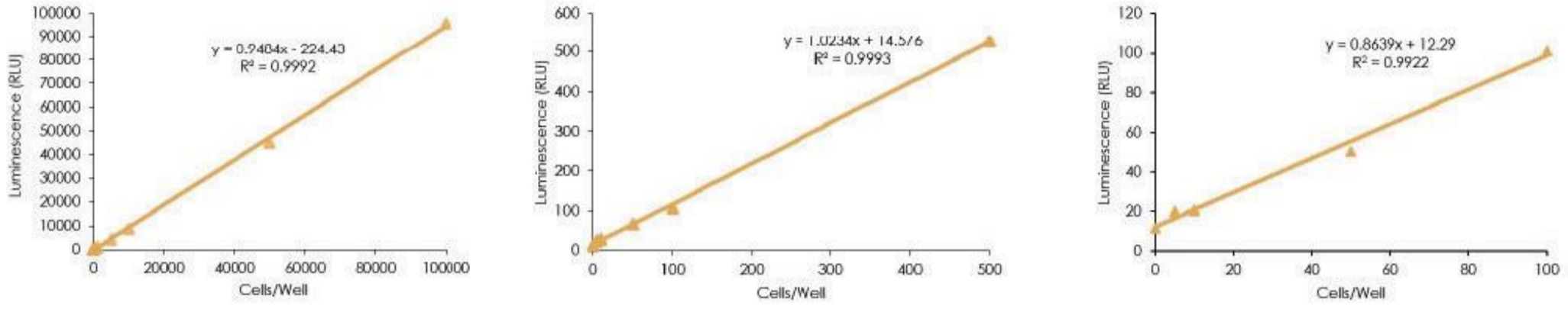
### Product Features

- Simple operation: Single-component, lysis within 10 minutes. Suitable for high-throughput detection.
- High sensitivity: As few as 5 cells detection.
- Good stability: Half-life of up to 3 hours; maintains good linearity even after being stored at room temperature for 7 days.
- Wide linear range: Shows good linearity for cell numbers ranging from 5 to 100,000 cells.

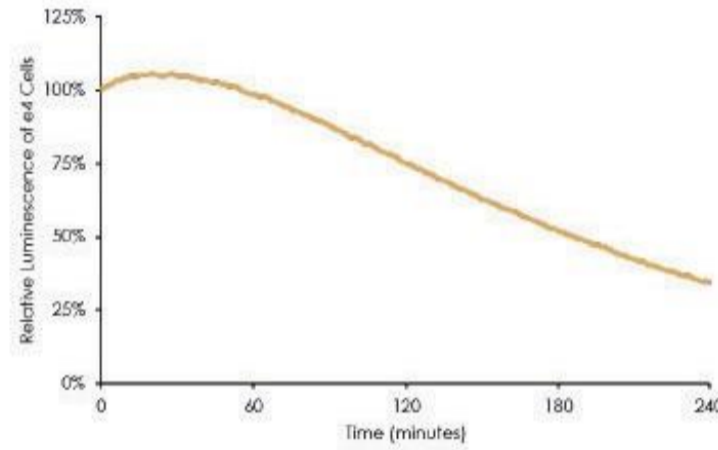


## Experimental Data

Wide linear range: Capable of detecting 5-100,000 cells, with high sensitivity allowing detection of as few as 5 cells.



Stable performance with up to 3 hours half-life



### Application Example - NK Cell Cytotoxicity Assay

NK cells were used as effector cells, while K562 cells were used as target cells. Effector cells and target cells were co-cultured at effector-to-target ratios of 20:1, 10:1, 5:1, and 1:1. After 24 hours of co-culture, the viability of cells in each group was assessed to calculate the cytotoxicity rate.

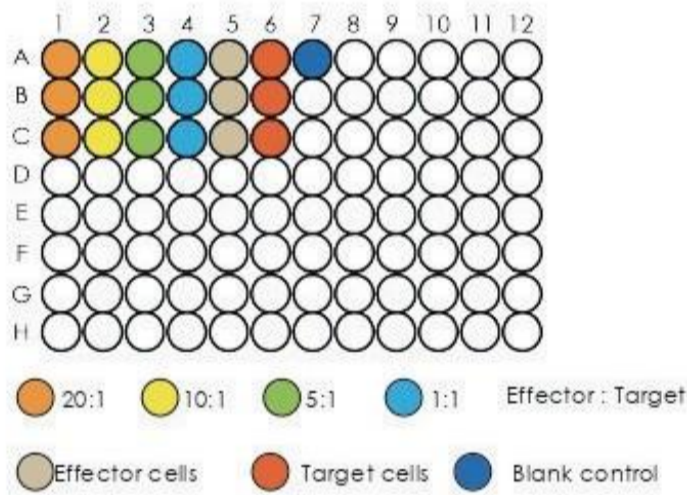
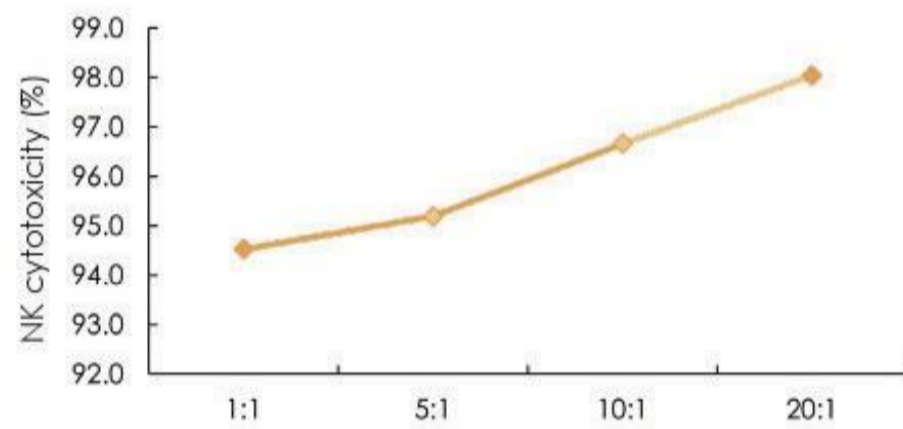
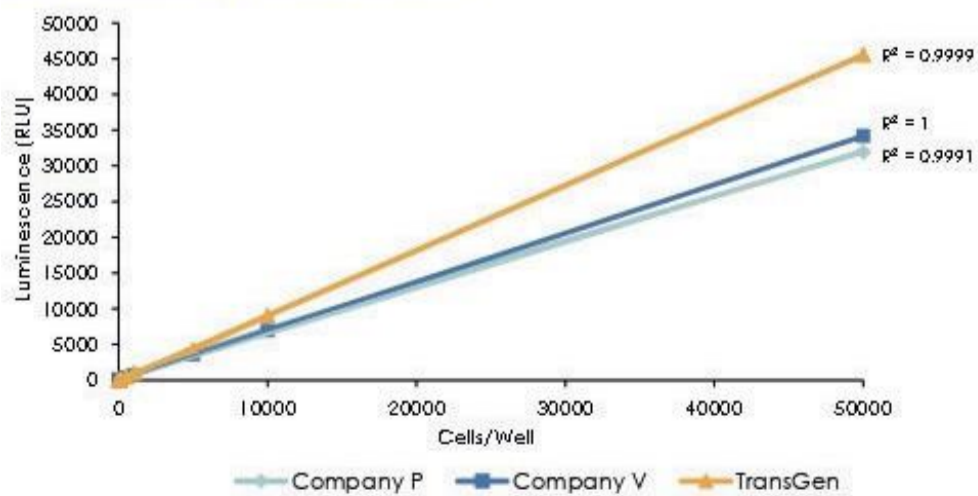


Illustration of the procedure

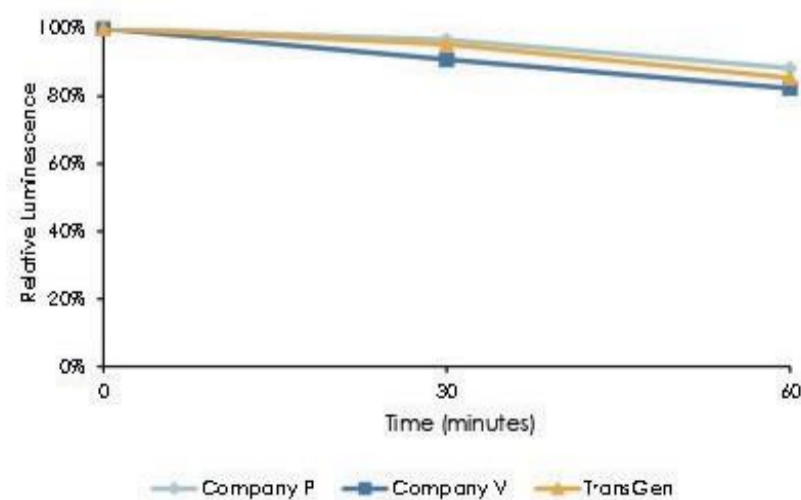


$NK \text{ cytotoxicity (\%)} = [1 - (\text{Luminescence value of Effector-Target well} - \text{Luminescence value of effector well}) / \text{Luminescence value of target well}] \times 100\%$

Fluorescence values are higher within the range of 5-50,000 cells, showing good linearity.



Stable performance, with a long half-life.





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