

Product Contents

Product	Volume
NanoSpark™ GROW-NK Soluble Activator	1 mL

NanoSpark GROW-NK Soluble Activator is provided in 1 mL vials. The activator is suspended in phosphate buffered saline glycerol. Store at -80 °C long-term. Once thawed, store at 4 °C protected from light for up to one month.

Description

Nanotein’s NanoSpark GROW-NK Soluble Activator is engineered to activate and expand NK cell populations. NanoSpark GROW-NK Soluble Activator is a self-assembling protein nanoparticle with anti-CD2 and anti-NKp46 antibodies conjugated to the surface. The proprietary biophysical combination of anti-CD2 and anti-NKp46 antibodies on the nanoparticle surface of GROW-NK leads to activation and expansion of NK cells. NanoSpark GROW-NK Soluble Activator is designed for use with cytokine-supplemented NK cell expansion medium.

Applications

Nanotein’s NanoSpark GROW-NK Soluble Activator is intended for *ex vivo* activation and expansion of NK cells.

Recommended Materials Not Provided

The following materials and equipment are recommended for use with NanoSpark GROW-NK Soluble Activator.

- Fresh or cryopreserved PBMC derived NK cells (StemCell Cat. #70036)
- CellGenix GMP SCGM Serum-Free Medium (CellGenix Cat. #20802-0500)
- Recombinant Human IL-2 (StemCell Cat. #78145)
- Recombinant Human IL-12 (StemCell Cat.# 78027)
- Recombinant Human IL-15 (StemCell Cat. #78031)
- Recombinant Human IL-18 (Bio-Techne Cat. #9124-IL/CF)
- Recombinant Human IL-21 (StemCell Cat. #78082)
- Human Platelet Lysate, Fibrinogen-Depleted, Xeno-Free (StemCell Cat. #200-0361)
- Sterile culture vessels
- Flow Cytometer
- Fluorophore-conjugated antibodies for flow cytometer characterization

Recommended Cytokine Combinations

Results	Combination
Optimal:	IL-2, IL-15, (35 ng/mL ea.) IL-21 (10 ng/mL)
Standard:	IL-2, IL-15 (35 ng/mL ea.)
Functional:	IL-2 (35 ng/mL)

Protocol

The following is a general protocol for using NanoSpark GROW-NK Soluble Activator. Optimization may be necessary depending on your experimental objectives and donor NK cell source/quality.

Fresh cells

1. Day 0 – Seeding
 - a. Exchange NK cells into culture media.
 - b. Count cells & seed at 7×10^5 cells/mL in culture media.
2. Day 0 – Activation
 - a. To activate and expand cells, add the appropriate amount of NanoSpark GROW-NK Soluble Activator for every mL of cell suspension as shown below:

Expansion Condition	GROW-NK Amount / mL
5% Serum:	5 μ L one time 1 μ L weekly dosing
No Serum:	20 μ L

- b. Add one of the recommended cytokine combinations to culture media.
 - c. Incubate cells at 37 °C and 5% CO₂ in a humidified incubator.
3. Day ~5-7 – Transduction (OPTIONAL)
 - a. Typical NK cell viral transductions occur within this window, however, optimization with cell number, source, and viability as key variables may be necessary.
 4. Cell Expansion & Maintenance
 - a. **Ensure activator is in culture media for at least 5 days (up to 10 days).**
 - b. **Redose activator (OPTIONAL) on days ~7, 14, 21 for greater expansion and higher cytotoxicity.**
 - c. Every 2-3 days monitor for viability & density adjustment.
 - i. Maintain cells between 5×10^5 cells/mL and 2×10^6 cells/mL.
 - ii. After a split/dilution aim for 5×10^5 cells/mL.
 - d. Add fresh culture medium supplemented with one of the recommended cytokine combinations to the appropriate cell density for your specific application.
 - e. Incubate cells at 37 °C and 5% CO₂ in a humidified incubator.

- f. Repeat these maintenance steps until the desired cell number is reached.

NOTE: Add fresh media with cytokines every 3-5 days.

Additional Notes & Insights

1. Donor-to-donor variation has been observed, particularly for NK cells from

younger individuals versus older, which may require additional optimization.

2. Protocol has been optimized for static culturing techniques. Non-static may require additional optimization.
3. For cryopreserved donor cells, some may require an overnight recovery period post-thaw before activation for optimal viability & expansion.

Example Data:

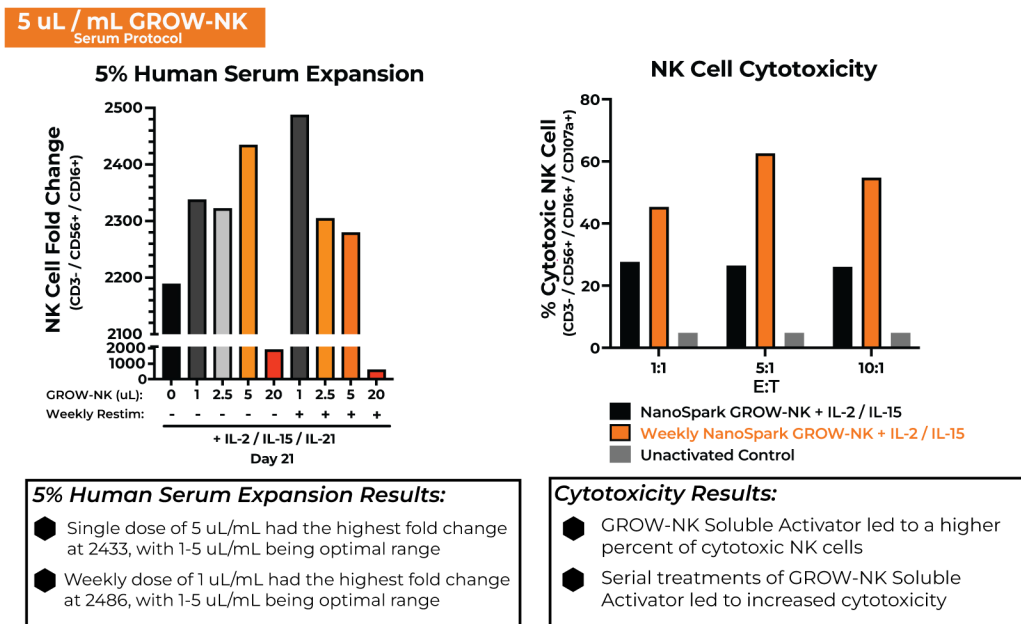
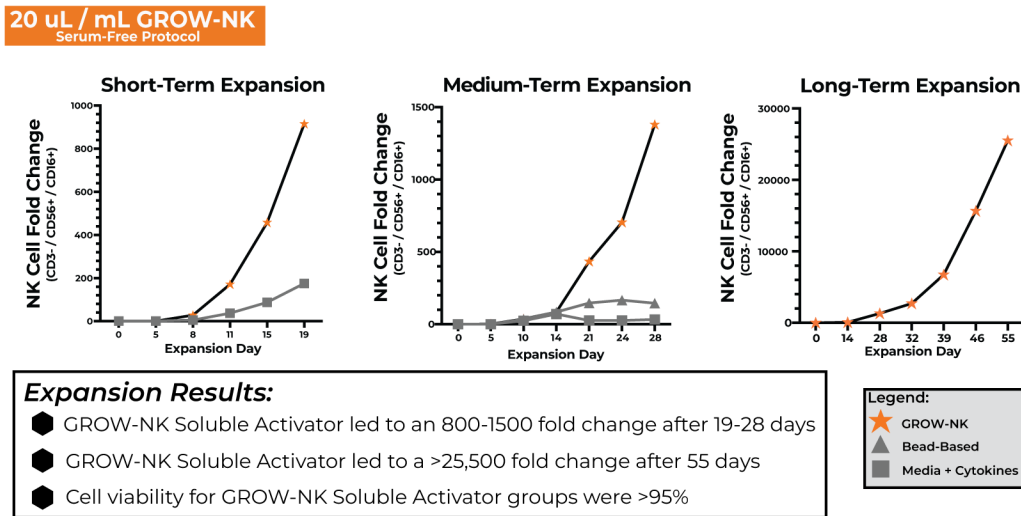


Figure 1: Fold Change and Cytotoxicity of NK Cells. NanoSpark GROW-NK Soluble Activator was cultured in CellGenix GMP SCGM with and without serum supplemented with the designated cytokines. Cells were expanded, labeled according to the following staining: CD3⁺, CD16⁺, CD56⁺, and NKp46⁺ using

fluorescent antibodies, and analyzed on a flow cytometer. For the cytotoxicity assay, day 28 expanded effector NK cells were mixed with target K562 cells at 1:1, 5:1, and 10:1 effector to target ratios and incubated for 4 hours. Cells were labeled according to the following staining: CD3⁺, CD16⁺, and CD107a⁺ using fluorescent antibodies. Expansion with NanoSpark GROW-NK Soluble Activator enhances total cell viability and stimulates expansion of NK cells while promoting their cytotoxicity.

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