NANOTEIN TECHNOLOGIES
www.nanoteintech.com
Toll Free +1 (866) 828-2355

NanoSpark™ GROW-NK Soluble Activator

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 (35 ng/mL) IL-12, IL-18, IL-21 (10 ng/mL ea.)
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 – Activation
    a. Exchange NK cells into culture media.
    b. Count cells & seed at 7 x 10^5 cells/mL in culture media.
    c. To activate and expand cells, add 20 µL of NanoSpark GROW-NK Soluble Activator for every mL of cell suspension (e.g., 160 µL of Activator for 8 mL of cell suspension).
    d. Add one of the recommended cytokine combinations to culture media.
    e. Incubate cells at 37 °C and 5% CO_2 in a humidified incubator.
2. 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.
3. 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 x 10^5 cells/mL and 2 x 10^6 cells/mL.
        ii. After a split/dilution aim for 5 x 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% CO_2 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:

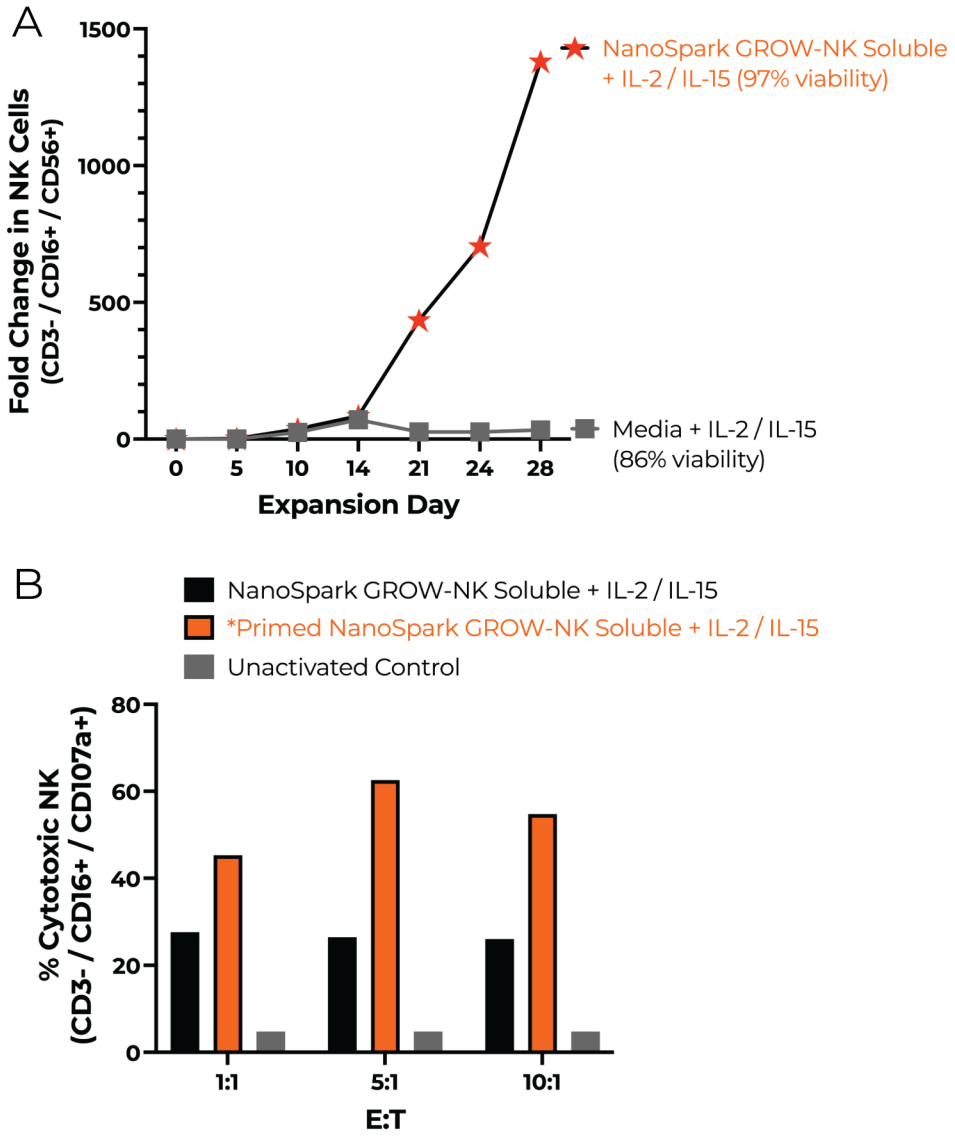

Figure 1: Fold Change and Cytotoxicity of NK Cells. NanoSpark GROW-NK Soluble Activator was cultured in CellGenix GMP SCGM (xeno-free and serum-free) supplemented with IL-2 and IL-15. **A)** Cells were expanded for 28 days and analyzed on a flow cytometer on days 0, 5, 10, 14, 21, 24 and 28. Cells were labeled according to the following staining: $CD3^-$, CD16+, CD56+, and NKp46+ using fluorescent antibodies. **B)** 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. *Primed cells were stimulated multiple times with GROW-NK. Expansion with NanoSpark GROW-NK Soluble Activator enhances total cell viability and stimulates expansion of NK cells while promoting their cytotoxicity.

Legal Notices

Use of this product is subject to, and constitutes acceptance of, the following terms and conditions.

The product is licensed to customer for internal research use only. Customer shall not (a) sell, distribute, rent, lease, lend, sublicense, or otherwise commercialize the product or any accompanying materials; (b) use the product for any therapeutic, diagnostic or other clinical purposes; (c) reverse engineer the product or any accompanying materials; (d) publicly release or disclose the results of, development, or evaluation of the product; or (e) remove or alter any proprietary notices or labels on or in the product or any accompanying materials.

THE PRODUCT AND ANY ACCOMPANYING MATERIALS ARE PROVIDED ON AN "AS IS" BASIS WITHOUT ANY REPRESENTATIONS OR WARRANTIES OF ANY KIND. NANOTEIN AND ITS LICENSORS DISCLAIM ANY AND ALL WARRANTIES AND REPRESENTATIONS (EXPRESS OR IMPLIED, ORAL OR WRITTEN) WITH RESPECT TO THE PRODUCT AND ANY ACCOMPANYING MATERIALS, WHETHER ALLEGED TO ARISE BY OPERATION OF LAW, BY REASON OF CUSTOM OR USAGE IN THE TRADE, BY COURSE OF DEALING OR OTHERWISE, INCLUDING ANY AND ALL (A) WARRANTIES OF MERCHANTABILITY, (B) WARRANTIES OF FITNESS OR SUITABILITY FOR ANY PURPOSE (WHETHER OR NOT NANOTEIN OR ITS LICENSORS KNOW, HAVE REASON TO KNOW, HAVE BEEN ADVISED OR ARE OTHERWISE AWARE OF ANY SUCH PURPOSE), AND (C) WARRANTIES OF NONINFRINGEMENT OR CONDITION OF TITLE. CUSTOMER ACKNOWLEDGES AND AGREES THAT IT HAS NOT RELIED ON ANY WARRANTIES REGARDING THE PRODUCT OR ACCOMPANYING MATERIALS, THAT IT HAS READ AND UNDERSTOOD ALL DOCUMENTATION PROVIDED WITH THE PRODUCT, AND THAT CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF THE NANOTEIN MATERIALS ARE SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION. CUSTOMER HEREBY ASSUMES ALL RISK ASSOCIATED WITH USE OF THE PRODUCT AND/OR ACCOMPANYING MATERIALS TO THE FULLEST EXTENT PERMITTED BY LAW.