

Product Contents

Product	Volume
NanoSpark™ STEM-T Soluble T-Cell Activator	1 mL

NanoSpark STEM-T Soluble T-Cell 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 STEM-T Soluble T Cell Activator is engineered to activate and expand an enriched human T lymphocyte population. NanoSpark STEM-T Soluble T Cell Activator is a self-assembling protein nanoparticle with anti-CD3 and anti-CD28 antibodies conjugated to the surface. The proprietary biophysical combination of anti-CD3 and anti-CD28 antibodies on the nanoparticle surface of STEM-T leads to strong primary and co-stimulatory signals that uniquely activate and expand T cells. NanoSpark STEM-T Soluble T Cell Activator is designed for use with cytokine-supplemented T cell expansion medium.

Applications

Nanotein’s NanoSpark STEM-T Soluble T Cell Activator is intended for *ex vivo* activation and expansion of CD3⁺ T Lymphocytes or human resting T cells from peripheral blood mononuclear cells (PBMCs).

Recommended Materials Not Provided

The following materials and equipment are recommended for use with NanoSpark STEM-T Soluble T-Cell Activator.

- Fresh or cryopreserved CD3⁺ T Lymphocytes or PBMCs (StemCell Cat. #70024 or 70025)
- Xeno-free T-Cell Expansion Media
 - StemCell Cat. #10981) OR
 - CellGenix GMP TCM (Sartorius Cat. # 20814-0500) OR
 - PRIME-XV T Cell Expansion XSFM (Irvine Scientific Cat. # 91141)
- Recombinant Human IL-7 (StemCell Cat. #78053)
- Recombinant Human IL-15 (StemCell Cat. #78031)
- Recombinant Human IL-2 (StemCell Cat. #78036)
- Human Serum (SigmaAldrich Cat. #H3667)
- Sterile culture vessels
- Flow Cytometer
- Fluorophore-conjugated antibodies for flow cytometer characterization

Recommended Cytokine Combinations

Results	Combination
Optimal:	IL-7, IL-15, (10 ng/mL ea.)
Functional:	IL-2 (20 ng/mL)

Protocol

The following is a general protocol for using NanoSpark STEM-T Soluble T Cell Activator. Optimization may be necessary depending on your experimental objectives.

1. Day 0 – Seeding
 - a. Exchange fresh or cryopreserved CD3⁺ T cells or PBMCs into culture media.
 - b. Count cells & seed at 1 x 10⁶ cells/mL in culture media.
2. Day 0 – Activation
 - a. To activate cells, add the appropriate amount of NanoSpark STEM-T Soluble T Cell Activator for every mL of cell suspension as shown below:

Expansion Condition	STEM-T Amount / mL
5% Serum:	1-3 μL
No Serum:	8-10 μL
 - 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. Transduction (OPTIONAL)
 - a. 24-72 hours after activator addition, apply viral vector for ~24-48 hours.
4. Electroporation (OPTIONAL)
 - a. 72 hours after activator addition, electroporate according to manufacturer’s instructions.
 - b. Add cells to media containing 5% human serum for better recovery.
5. Cell Expansion & Maintenance
 - a. Ensure activator is in culture media (conditioned or fresh) for at least ~72 hours (up to 6 days) for optimal expansion.**
 - b. Every 2-3 days monitor and/or count the cells for viability & density adjustment.
 - c. Add fresh culture medium supplemented with one of the recommended cytokine combinations to the appropriate cell density for your specific application.
 - d. Incubate cells at 37 °C and 5% CO₂ in a humidified incubator.
 - e. Repeat these maintenance steps until the desired cell number is reached or up to 14 days after initial activation.

NOTE: Be sure to add fresh culture media supplemented with cytokines every 2-3 days.

Example Data

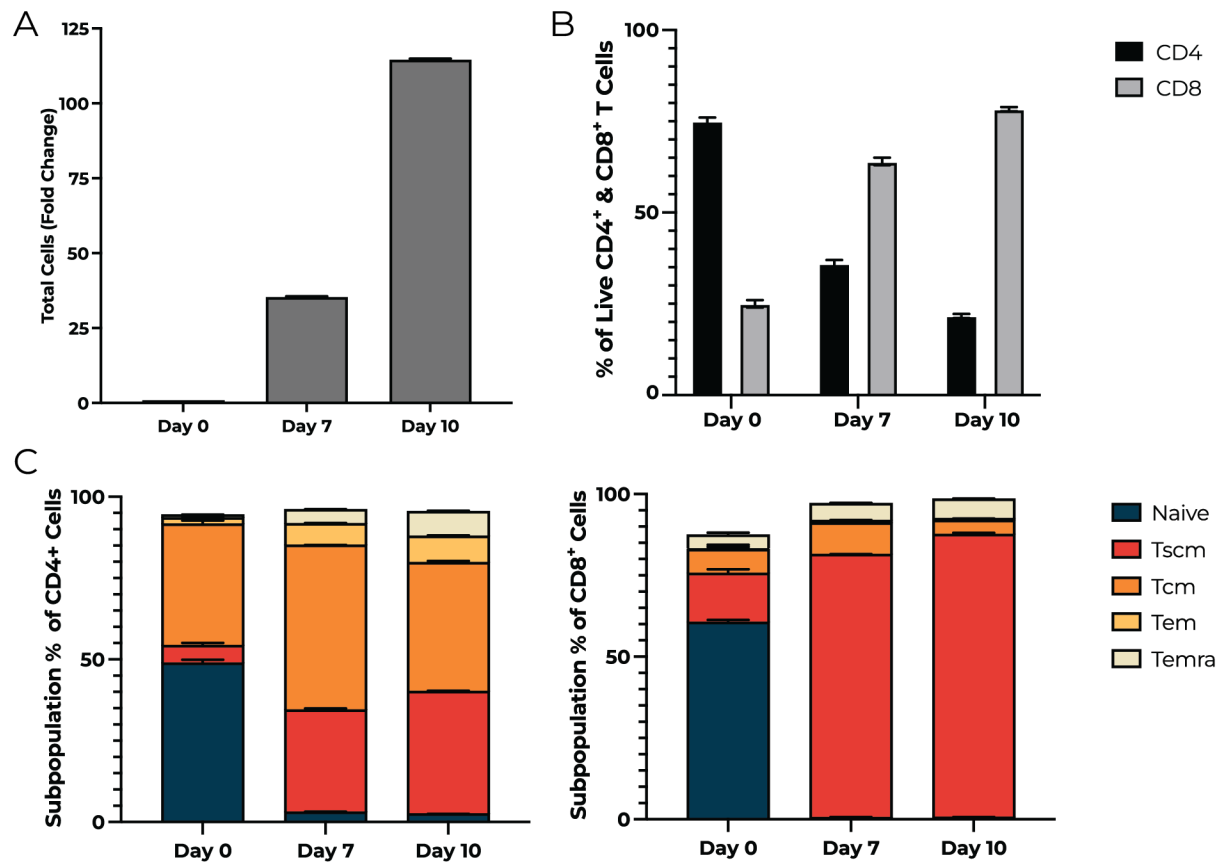


Figure 1. A) Fold Change of CD3⁺ Cells, B) % of CD4⁺ & CD8⁺ Cells, C) CD4⁺ & CD8⁺ T-Cell Phenotype Percentage. NanoSpark STEM-T Soluble T-Cell Activator was cultured in StemCell's ImmunoCult-XF T Cell Expansion Medium (xeno-free) supplemented with IL-7 and IL-15. Cells were expanded for 10 days and analyzed on a flow cytometer on days 0, 7, and 10. Cells were labeled with CD4⁺, CD8⁺, CD45RA, CCR7, and CD95 fluorescent antibodies (Schmueck-Henneresse et al 2017). Expansion with NanoSpark STEM-T Soluble T-Cell Activator enhances total cell viability and stimulates expansion of CD8⁺ T-Cells and promotes the stem-like phenotypes of CD4⁺ and CD8⁺ T-Cells: T_{naive}, T_{scm}, and T_{cm}.

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