

### 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 to be used 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<sup>+</sup> 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<sup>+</sup> T Lymphocytes or PBMCs (StemCell Cat. #70024 or 70025)
- Xeno-free T-Cell Expansion Media (StemCell Cat. #10981)
- Recombinant Human IL-2 (StemCell Cat. #78036)
- Sterile culture vessels
- Flow Cytometer
- Fluorophore-conjugated antibodies for flow cytometer characterization.

### 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.

### Fresh cells

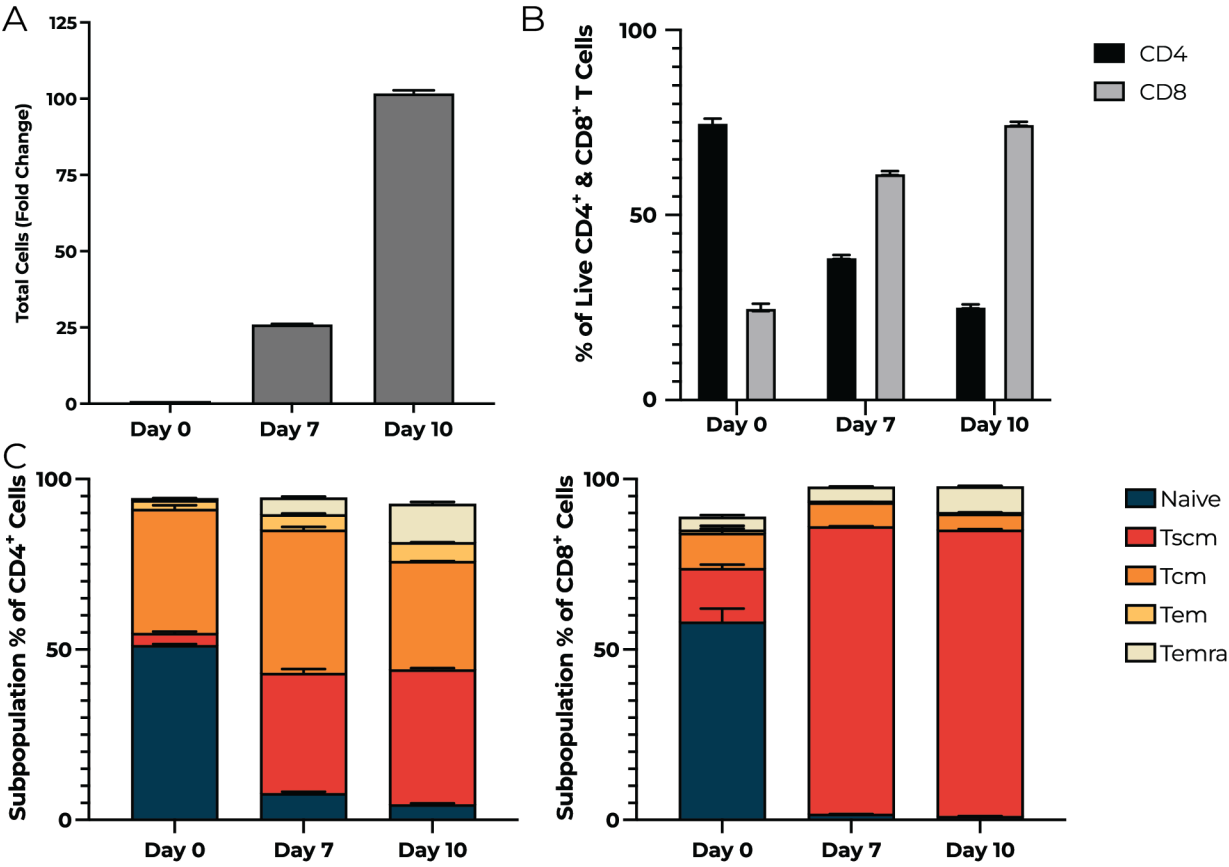
1. Day 0 – Activation
  - a. Exchange CD3<sup>+</sup> T cells or PBMCs into culture media.
  - b. Count cells & seed at 1 x 10<sup>6</sup> cells/mL in culture media.
  - c. To activate cells, add 10 µL of NanoSpark STEM-T Soluble T Cell Activator for every mL of cell suspension. (e.g., 80 µL of Activator for 8 mL of cell suspension).
  - d. Add IL-2 to culture media at 20 ng/mL final concentration.
  - e. Incubate cells at 37 °C and 5% CO<sub>2</sub> overnight in a humidified incubator.
2. Day 1 – Transduction (OPTIONAL)
  - a. 24 hours after activator addition, apply viral vector for ~24-48 hours.
3. Cell Expansion & Maintenance
  - a. **Ensure activator is in culture media (conditioned or fresh) for at least ~72 hours (up to 6 days).**
  - b. Every 2-3 days monitor and/or count the cells for viability & density adjustment.
  - c. Add fresh culture medium supplemented with 20 ng/mL IL-2 to the appropriate cell density for your specific application.
  - d. Incubate cells at 37 °C and 5% CO<sub>2</sub> 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 IL-2 every 3 days. For optimization in your specific setup, **consider using activator between 5-10 µL per mL.**

### Cryopreserved Cells

1. Day -1
  - a. Thaw and exchange CD3<sup>+</sup> T cells or PBMCs into culture media.
  - b. Count cells & seed at 1 x 10<sup>6</sup> cells/mL in culture media.
  - c. Incubate cells at 37 °C and 5% CO<sub>2</sub> overnight in a humidified incubator.
2. Day 0 [Follow Steps 1c – 3e under “Fresh cells” above].

**Example Data**



**Figure 1. A) Fold Change of CD3+ Cells, B) % of CD4+ & CD8+ Cells, C) CD4+ & CD8+ T-Cell Subpopulation Percentages.** NanoSpark STEM-T Soluble T-Cell Activator was cultured in StemCell's ImmunoCult-XF T Cell Expansion Medium (xeno-free) supplemented with IL-2. 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<sub>naive</sub>, T<sub>scm</sub>, and T<sub>cm</sub>.

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