

**Product Contents**

| Product   | Volume |
|---|--------|
| <b>NanoSpark™ STEM-T Soluble T-Cell Activator</b> | 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<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
  - o StemCell Cat. #10981) OR
  - o CellGenix GMP TCM (Sartorius Cat. # 20814-0500) OR
  - o 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 G-Rex6M (10 cm<sup>2</sup> cell growth area) (Wilson Wolf P/N 80660M)
- Flow Cytometer
- Fluorophore-conjugated antibodies

**Recommended Cytokine Combinations**

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

**Protocol**

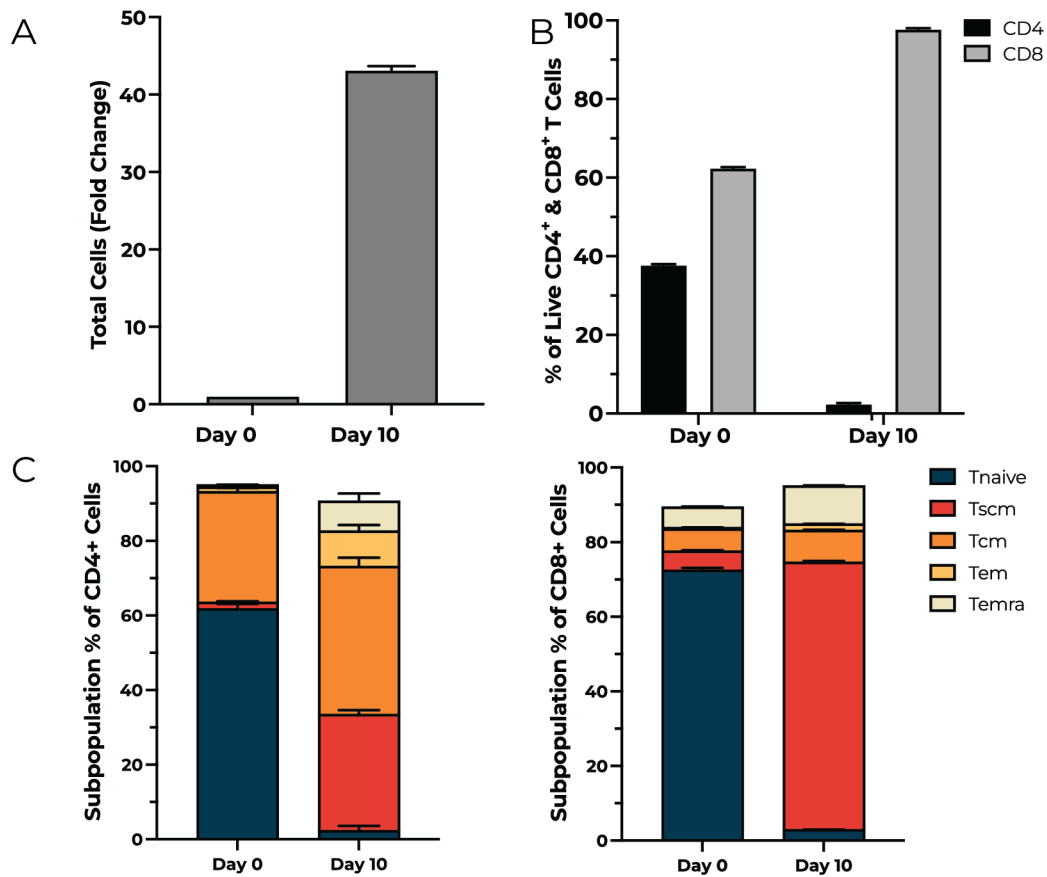
The following is a general protocol for using NanoSpark STEM-T Soluble T Cell Activator. Optimization may be necessary depending on your experimental objectives.

1. Day 0 – Seeding
  - a. Exchange CD3<sup>+</sup> T cells or PBMCs into culture media.
  - b. Count cells & seed at 7 x 10<sup>5</sup> cells/cm<sup>2</sup> 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 |
|---------------------|--------------------|
| <b>5% Serum:</b>    | 1-3 μL             |
| <b>No Serum:</b>    | 8-10 μL            |
  - b. Add one of the recommended cytokine combinations to culture media.
  - c. Incubate cells at 37 °C and 5% CO<sub>2</sub> in a humidified incubator.
3. Transduction (OPTIONAL)
  - a. 24 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% CO<sub>2</sub> in a humidified incubator.
  - e. Repeat these maintenance steps until the desired cell number is reached or up to 10 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<sup>+</sup> Cells, B) % of CD4<sup>+</sup> & CD8<sup>+</sup> Cells, C) CD4<sup>+</sup> & CD8<sup>+</sup> 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 in a G-rex6M and analyzed on a flow cytometer on days 0, and 10. Cells were labeled with CD4<sup>+</sup>, CD8<sup>+</sup>, 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<sup>+</sup> T-Cells and promotes the stem-like phenotypes of CD4<sup>+</sup> and CD8<sup>+</sup> T-Cells: T<sub>naive</sub>, T<sub>scm</sub>, and T<sub>cm</sub>.

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