

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
 - o StemCell Cat. #10981) OR
 - o CellGenix GMP TCM (Sartorius Cat. # 20814-0500) OR
 - o PRIME-XV T Cell Expansion XFSM (Irvine Scientific Cat. # 91141) OR
 - o TheraPEAK® T-VIVO® Medium (Lonza BP12-970Q) OR
 - o TheraPEAK™ X-VIVO™ 15 Medium (Lonza BPO4-744Q)
- 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)
- CTS™ Immune Cell SR (Thermo Cat. # A2596101)
- Sterile culture vessels
- Flow Cytometer
- Fluorophore-conjugated antibodies for flow cytometer characterization

Recommended Expansion Conditions

Best Protocols	Combination	Serum/Replacement	STEM-T (µL/mL)
Maximize Speed (highest total cells per time)	IL-2, IL-7, IL-15 (10 ng/mL ea.)	5% CTS™ Immune Cell Serum Replacement (SR)	8 (8-10)
Maximize Stemness	IL-7, IL-15 (10 ng/mL ea.)	Serum Free (SF)	8 (8-10)
Other Functional Combos	IL-2, IL-7, IL-15 (10 ng/mL ea.)	5% Human Serum	5 (2-5)
	IL-2 (20 ng/mL) OR IL-7, IL-15 (10 ng/mL ea.)	SF, 5% SR, or 5% Serum	8 (2-10)

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 above in the recommended expansion conditions table.
 - i. Add the corresponding cytokine combination to culture media.
 - b. 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 or SR 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

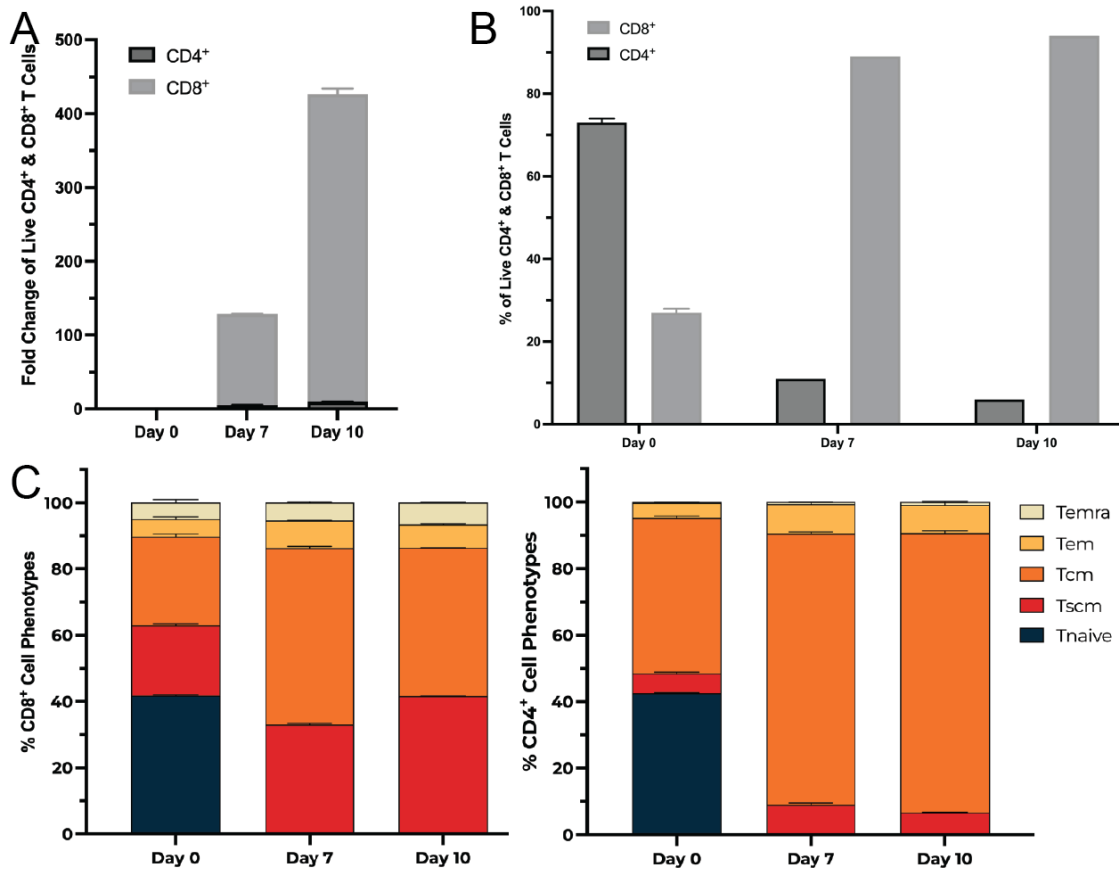
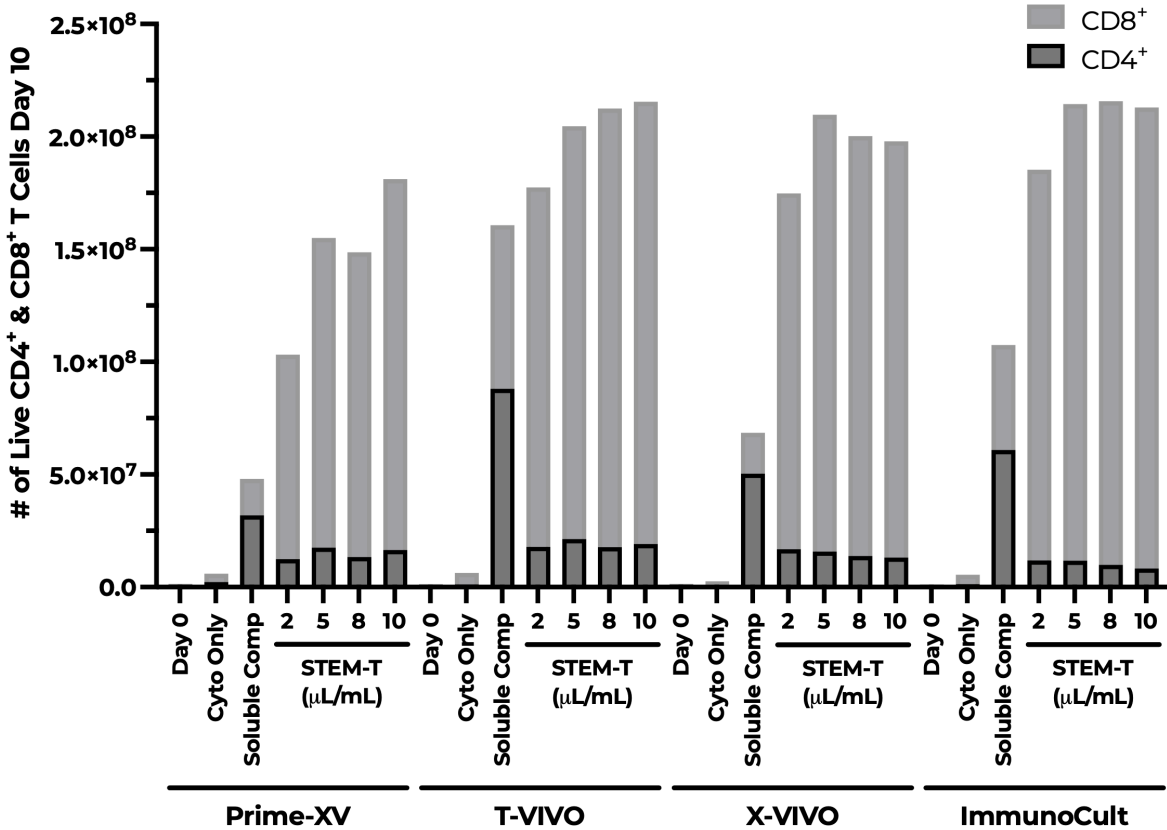
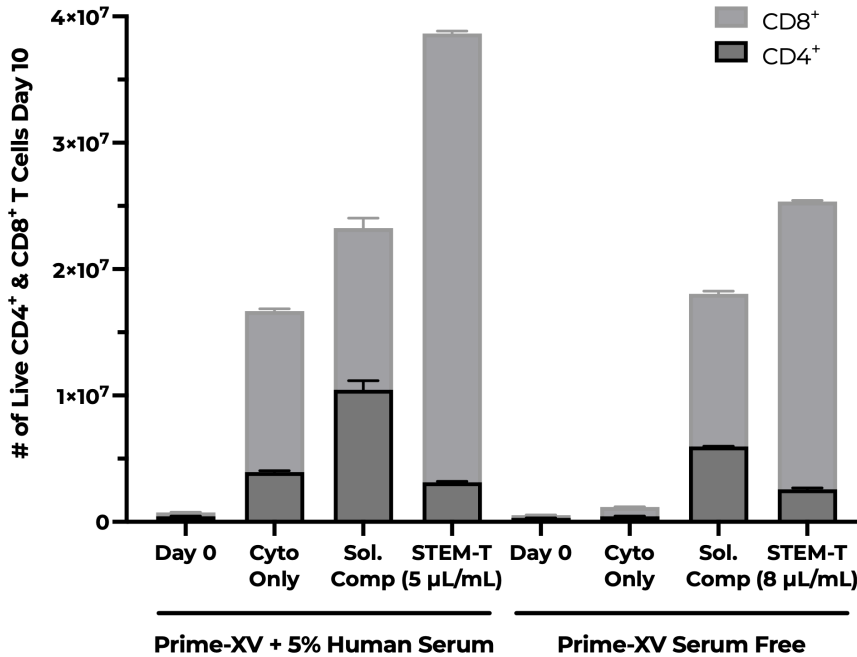


Figure 1 - Maximize Stemness. A) Fold Change of CD3⁺ Cells, B) % of CD4⁺ & CD8⁺ Cells, C) CD8⁺ & CD4⁺ T-Cell Phenotype Percentage. NanoSpark STEM-T Soluble T-Cell Activator was cultured in StemCell's ImmunoCult-XF T Cell Expansion Medium (xeno-free) supplemented with IL-7 and IL-15. Cells were expanded 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}.



***All with 5% Serum Replacement + IL-2, IL-7, IL-15 (10 ng/mL ea.)**

Figure 2 – Maximize Speed with Serum Replacement. NanoSpark STEM-T Soluble T-Cell Activator was compared to a common soluble activator (Soluble Comp) added to FujiFilm's Prime-XV, Lonza's T-VIVO & X-VIVO, and StemCell's ImmunoCult-XF T Cell Expansion Media supplemented with 10 ng/mL each of IL-2, IL-7, and IL-15 and 5% CTS™ Immune Cell Serum Replacement. Cells were expanded for 10 days and analyzed on a flow cytometer on days 0, 7 (not shown), 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 generates more T cells with a higher CD8⁺ T cell content than the Soluble Comp product. These representative data were collected from expansion of a single donor's peripheral blood pan-T cells.



*All with IL-2, IL-7, IL-15 (10 ng/mL ea.)

Figure 3 – Optimal High-Yield Human Serum and Serum Free Conditions. NanoSpark STEM-T Soluble T-Cell Activator was compared to a common soluble activator (Soluble Comp) added to FujiFilm’s Prime-XV Media supplemented with 10 ng/mL each of IL-2, IL-7, and IL-15 with and without 5% heat inactivated Human Serum. Cells were expanded for 10 days and analyzed on a flow cytometer on days 0, 7 (not shown), 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 generates more T cells with a higher CD8⁺ T cell content than the Soluble Comp product. These representative data were collected from expansion of a single donor’s peripheral blood pan-T cells.

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