NANOTEIN TECHNOLOGIES

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NanoSpark® STEM-T Soluble T Cell Activator Standard 7-10 Day Protocol

Product Contents

Product	Volume
NanoSpark™ STEM-T Soluble T-Cell Activator	2 mL

NanoSpark STEM-T Soluble T-Cell Activator is provided in 2 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
  - ImmunoCell Growth Medium (AkronBio AK9985-1000) OR
  - ImmunoCult (StemCell Cat. #10981) OR
  - CellGenix GMP TCM (Sartorius Cat. # 20814-0500) OR
  - PRIME-XV T Cell Expansion XSFM (Irvine Scientific Cat. # 91141) OR
- Recombinant Human IL-2 (AkronBio Cat. #AK8223-0100)
- Recombinant Human IL-7 (AkronBio Cat. #AK9842-0040)
- Recombinant Human IL-15 (AkronBio Cat. #AK9823-0040)
- Human AB Serum (AkronBio Cat. #AR1010-0100)
- 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)	Transduction Time (Hours)
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)	48 (24-48)
Maximize Stemness	IL-7, IL-15 (10 ng/mL ea.)	Serum Free (SF)	8 (8-10)	72
Other Functional Combos	IL-2, IL-7, IL-15 (10 ng/mL ea.)	5% Human Serum	5 (2-5)	48
	IL-2 (20 ng/mL) OR IL-7, IL-15 (10 ng/mL ea.)	SF, 5% SR, or 5% Serum	8 (2-10)	72

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 10^6 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% CO_2 in a humidified incubator.
3. **Transduction** (OPTIONAL)
   a. 24-72 hours after activator addition, apply viral vector for ~24-48 hours.
      i. For IL-2, IL-7, IL-15 Expansion apply viral vector 48 hours after activator addition
      ii. For IL-7 and IL-15 Expansion apply viral vector 72 hours after activator addition
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% CO_2 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

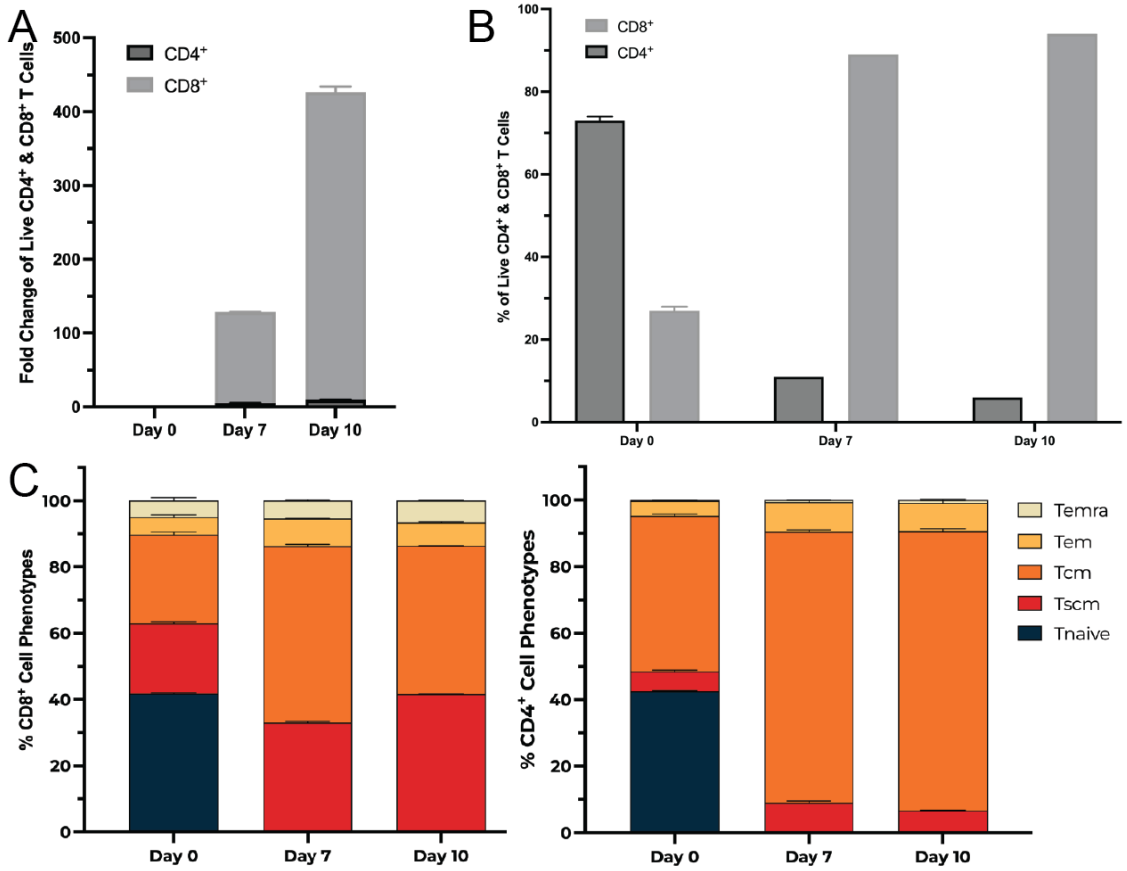

Figure 1 - Maximize Stemness. A) Fold Change of CD3⁺ Cells, B) % of CD4⁺ & CD8⁺ Cells, C) CD8⁺ & CD4⁺ T-Cell Phenotype Percentage. NanoSpark STEM-T Soluble T-Cell Activator was cultured in StemCell's ImmunoCult-XF T Cell Expansion Medium (xeno-free) supplemented with IL-7 and IL-15. Cells were expanded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

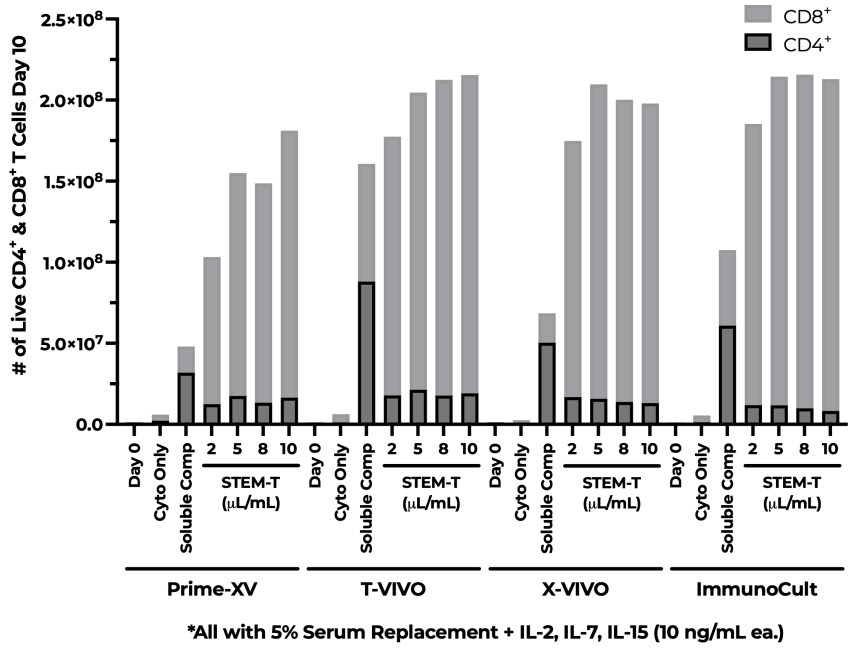

Figure 2 – Maximize Speed with Serum Replacement. NanoSpark STEM-T Soluble T-Cell Activator was compared to a common soluble activator (Soluble Comp) added to FujiFilm's Prime-XV, Lonza's T-VIVO & X-VIVO, and StemCell's ImmunoCult-XF T Cell Expansion Media supplemented with 10 ng/mL each of IL-2, IL-7, 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

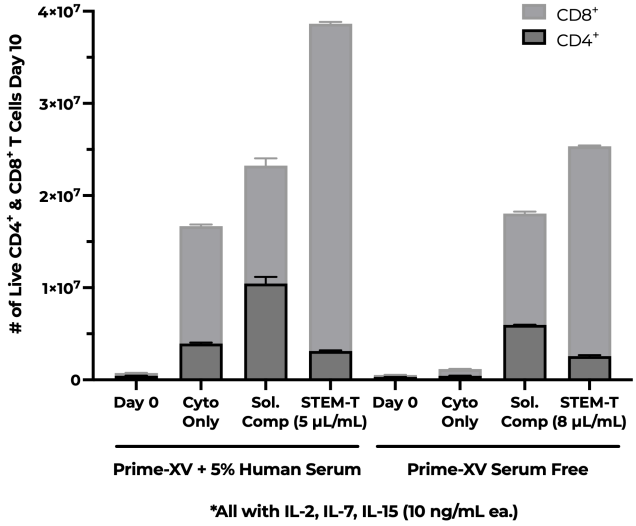

Figure 3 – Optimal High-Yield Human Serum and Serum Free Conditions. NanoSpark STEM-T Soluble T-Cell Activator was compared to a common soluble activator (Soluble Comp) added to FujiFilm's Prime-XV Media supplemented with 10 ng/mL each of IL-2, IL-7, and IL-15 with and without 5% heat inactivated Human 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.

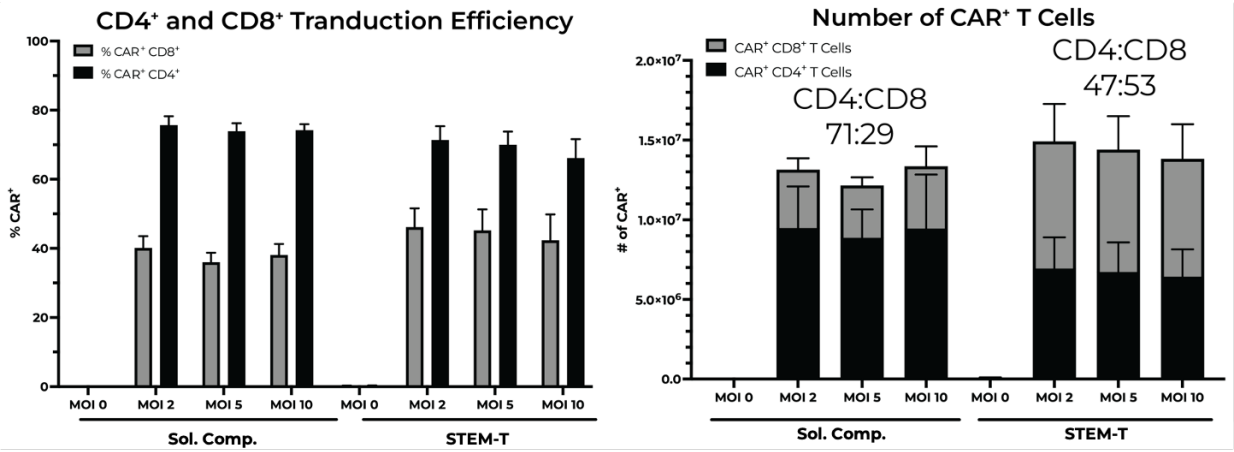

Figure 4 – CAR-T Cell Generation & 10-Day Expansion. NanoSpark STEM-T Soluble T-Cell Activator was compared to a common soluble activator (Soluble Comp) added to FujiFilm's Prime-XV Media supplemented with 10 ng/mL each of IL-2, IL-7, and IL-15 with and 5% CTS™ Immune Cell Serum Replacement. A third generation CAR construct: scFv(FMC63)-41BB-CD3ζ was transduced on Day 2. 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 CAR-T cells with a higher CD8⁺ T cell content than the Soluble Comp product. These data were collected from expansion of 3 unique donor's peripheral blood pan-T cells and averaged.

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