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

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

Applications

Nanotein’s NanoSpark EVEN-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 EVEN-T Soluble T-Cell Activator.

- * Fresh or cryopreserved CD3⁺ T Lymphocytes or PBMCs (StemCell Cat. #70024 or 70025)
- * CellGenix GMP TCM (Sartorius Cat. # 20814-0500)
- * PRIME-XV T Cell Expansion XSFM (Irvine Scientific Cat. # 91141)
- * TheraPEAK T-VIVO Cell Culture Medium (Lonza Cat.# BP12-970Q)
- * Recombinant Human IL-7 (StemCell Cat. #78053)
- * Recombinant Human IL-15 (StemCell Cat. #78031)
- * Sterile culture vessels
- * Flow Cytometer
- * Fluorophore-conjugated antibodies for flow cytometer characterization

Protocol

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

Fresh cells

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 4-10 µL of NanoSpark EVEN-T Soluble T Cell Activator for every mL of cell suspension ***MEDIA DEPENDENT (see Figure 1)**. (e.g., 32-100 µL of Activator for 8 mL of cell suspension).
 - b. Add IL-7 and IL-15 to culture media for a 10 ng/mL final concentration of each.
 - 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 and can be left in throughout the expansion.**
 - b. Every 2-3 days monitor and/or count the cells for viability & density adjustment.
 - c. Add fresh culture medium supplemented with 10 ng/mL IL-7 and 10 ng/mL IL-15 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 IL-7 and IL-15 every 3 days. For optimization in your specific setup, **consider using activator between 5 - 10 µL per mL (see Figure 1)*.**

*** Serum addition may further enhance percentage of CD4⁺ T cells – Optimization Required ***

*All Serum-Free

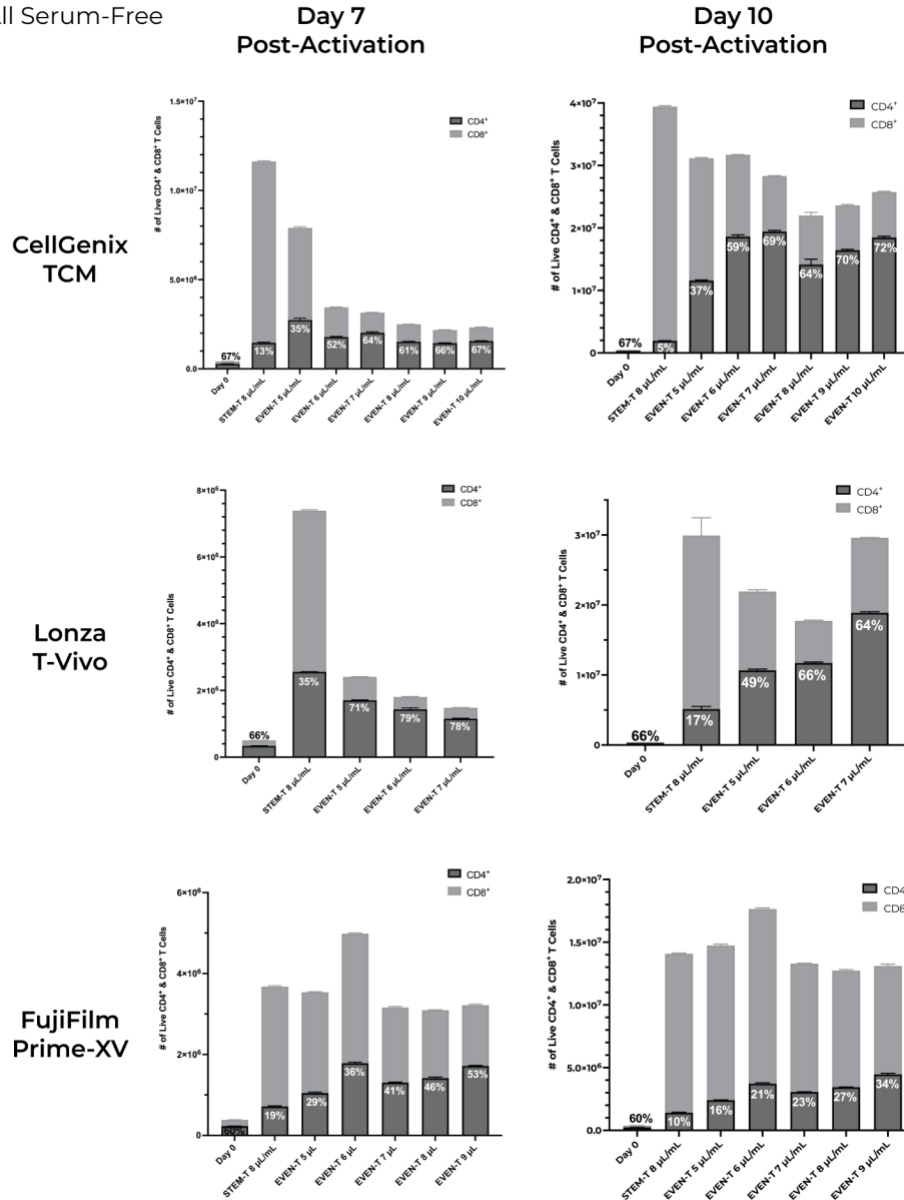


Figure 1. Increasing amounts of EVEN-T shifts the CD4⁺:CD8⁺ ratio towards CD4⁺s with media-dependent differences in functional windows. NanoSpark EVEN-T Soluble T-Cell Activator was added to CD3⁺ negatively selected human peripheral blood T cells cultured in CellGenix’s TCM, Lonza’s T-Vivo, or FujiFilm’s Prime-XV serum-free media supplemented with IL-7 and IL-15 and compared to NanoSpark STEM-T Soluble T-Cell Activator. 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 EVEN-T Soluble T-Cell Activator modulates the expansion to shift the expanded T cell profile towards CD4⁺ T cells in a dose-dependent manner, effectively allowing the user to select a CD4⁺:CD8⁺ T cell ratio at the outset of expansion.

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