

### 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 costimulatory 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
  - 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)
- CTST™ Immune Cell SR (Thermo Cat. # A2596101)
- Sterile culture vessels
- Flow Cytometer

- Fluorophore-conjugated antibodies for flow cytometer characterization

### Recommended Expansion Conditions

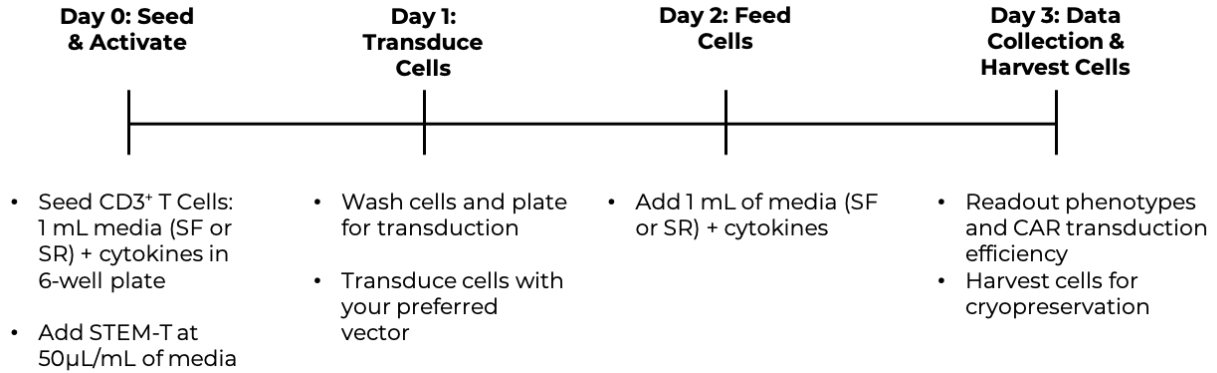
| Best Protocols          | Combination                      | Serum Replacement  | STEM-T (µL/mL) | Transduction Time (Hours) |
|-------------------------|----------------------------------|--|----------------|---------------------------|
| <b>24-hr Activation</b> | IL-2, IL-7, IL-15 (10 ng/mL ea.) | 5% CTST™ Immune Cell Serum Replacement ( <b>SR</b> ) OR Serum Free ( <b>SF</b> ) | 50             | 24                        |

### Protocol

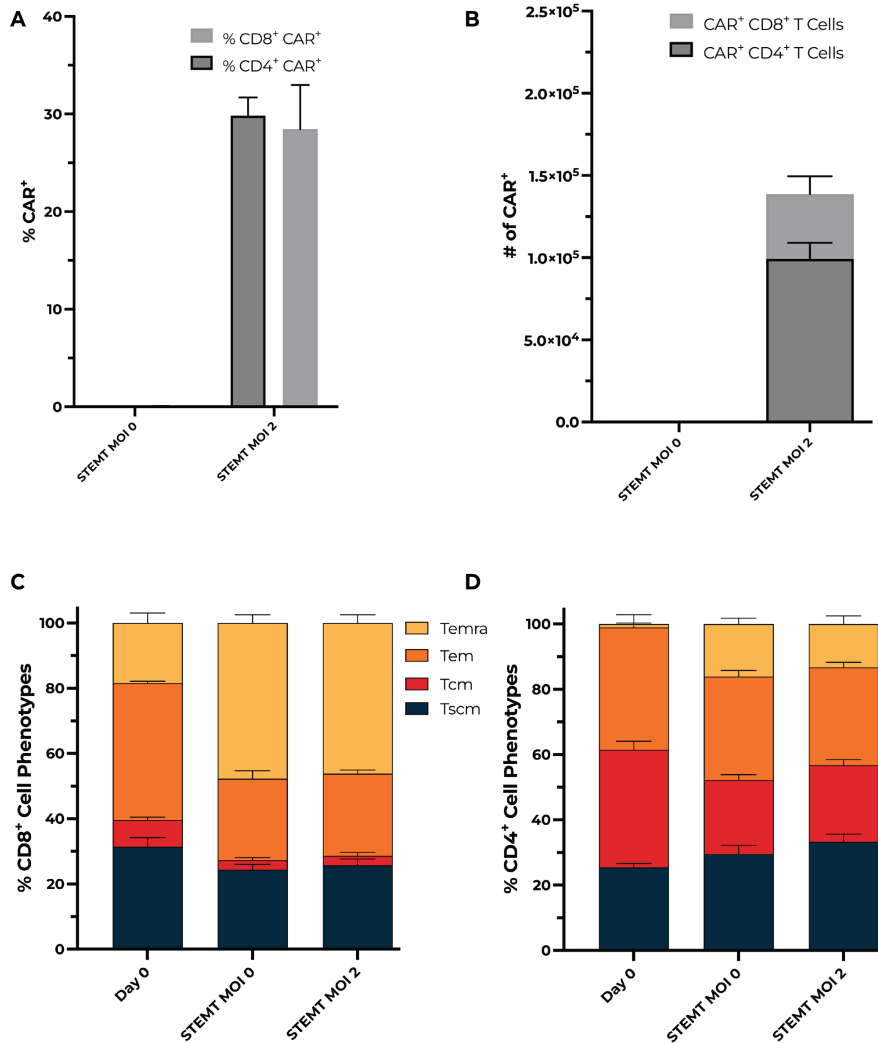
The following is a general protocol for using NanoSpark STEM-T Soluble T Cell Activator. Optimization may be necessary depending on your experimental objectives.

1. Day 0 – **Seeding**
  - a. Exchange fresh or cryopreserved CD3<sup>+</sup> T cells or PBMCs into culture media.
  - b. Count cells & seed at 2 x 10<sup>6</sup> cells/mL in culture media.
2. Day 0 – **Activation**
  - a. To activate cells, add 50 µL of NanoSpark STEM-T Soluble T Cell Activator for every mL of cell suspension as shown in the above table.
    - i. Add the corresponding cytokine combination to culture media.
  - b. Incubate cells at 37 °C and 5% CO<sub>2</sub> in a humidified incubator.
3. Day 1 – **Transduction**
  - a. 24 hours after activator addition, remove activator via centrifugation.
  - b. Then apply viral vector for 24 hours.
4. (OPTIONAL/Alternative) **Electroporation**
  - a. 24 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. Day 2 – **Cell Feeding**
  - a. Add fresh culture medium supplemented with cytokines to the appropriate cell density for your specific application.
  - b. Incubate cells at 37 °C and 5% CO<sub>2</sub> in a humidified incubator.
6. Day 3 – **Harvest Cells**
  - a. Harvest and wash cells for analysis, cryopreservation, and any future application(s).

### 3 Day Expansion Timeline



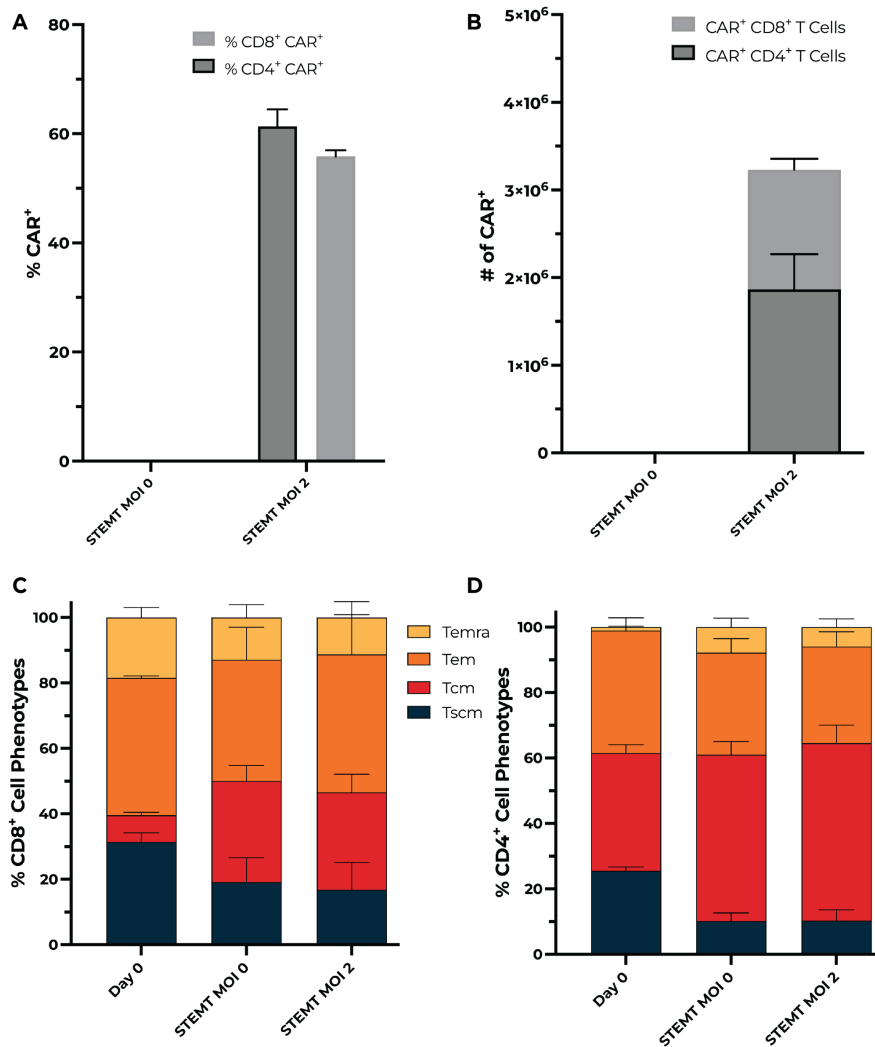
### Example Data



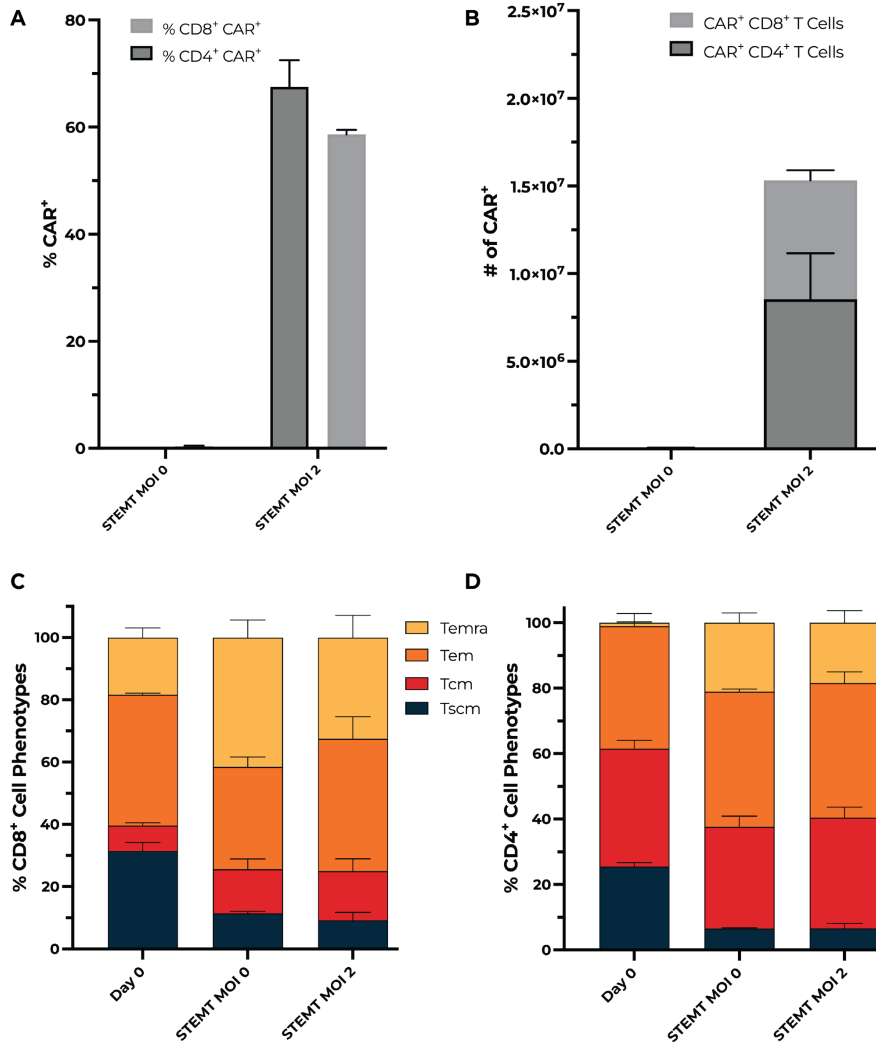
**Figure 1 – Day 3 Transduction A) Transduction Efficiency, B) Number of CAR<sup>+</sup> Cells, C) CD8<sup>+</sup> & CD4<sup>+</sup> T-Cell Phenotype Percentage.** NanoSpark STEM-T Soluble T-Cell Activator was cultured in Akron Bio's

ImmunoCell Growth medium (xeno-free) supplemented with IL-2, IL-7, and IL-15. Cells were expanded for 3 days and analyzed on a flow cytometer on days 0 and 3. Cells were labeled with CD4<sup>+</sup>, CD8<sup>+</sup>, CD45RA, and CCR7 fluorescent antibodies (Schmueck-Henneresse et al 2017). Expansion with NanoSpark STEM-T Soluble T-Cell Activator is versatile and can enhance total cell viability and stimulate expansion of CD8<sup>+</sup> T-Cells and promote the stem-like phenotypes of CD4<sup>+</sup> and CD8<sup>+</sup> T-Cells: T<sub>scm</sub>, and T<sub>cm</sub>.

**Example of Post-Day 3 Expansion “Follow-through” Results Below**



**Figure 2 – Day 7 Transduction A) Transduction Efficiency, B) Number of CAR<sup>+</sup> Cells, C) CD8<sup>+</sup> & CD4<sup>+</sup> T-Cell Phenotype Percentage.** NanoSpark STEM-T Soluble T-Cell Activator was cultured in Akron Bio’s ImmunoCell Growth medium (xeno-free) supplemented with IL-2, IL-7, and IL-15. Cells were expanded for 7 days and analyzed on a flow cytometer on days 0, 3, and 7. Cells were labeled with CD4<sup>+</sup>, CD8<sup>+</sup>, CD45RA, and CCR7 fluorescent antibodies (Schmueck-Henneresse et al 2017). Expansion with NanoSpark STEM-T Soluble T-Cell Activator is versatile and can enhance total cell viability and stimulate expansion of CD8<sup>+</sup> T-Cells and promote the stem-like phenotypes of CD4<sup>+</sup> and CD8<sup>+</sup> T-Cells: T<sub>scm</sub>, and T<sub>cm</sub>.



**Figure 3 – Day 10 Transduction A) Transduction Efficiency, B) Number of CAR<sup>+</sup> Cells, C) CD8<sup>+</sup> & CD4<sup>+</sup> T-Cell Phenotype Percentage.** NanoSpark STEM-T Soluble T-Cell Activator was cultured in Akron Bio's ImmunoCell Growth medium (xeno-free) supplemented with IL-2, IL-7, and IL-15. Cells were expanded for 10 days and analyzed on a flow cytometer on days 0, 3, 7 and Day 10. Cells were labeled with CD4<sup>+</sup>, CD8<sup>+</sup>, CD45RA, and CCR7 fluorescent antibodies (Schmueck-Henneresse et al 2017). Expansion with NanoSpark STEM-T Soluble T-Cell Activator is versatile and can enhance total cell viability and stimulate expansion of CD8<sup>+</sup> T-Cells and promote the stem-like phenotypes of CD4<sup>+</sup> and CD8<sup>+</sup> T-Cells: T<sub>scm</sub>, and T<sub>cm</sub>.

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