Synecta™ T1

Catalog No. BW-010407BE

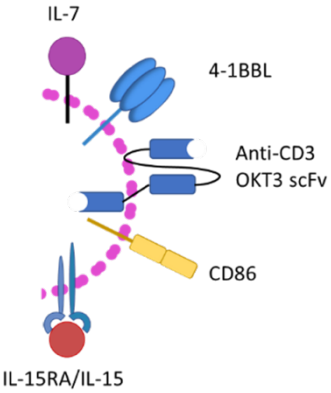
Contents

Product Contents

Product	Volume
Synecta™ T1	1 x 1 mL

1. Description

BlueWhale Bio®'s Synecta™ products are cell-derived nanoparticles engineered to express membrane-bound cytokines and co-stimulatory molecules. Synecta™ T1 activates and expands human T lymphocytes.



2. Applications

Synecta™ T1 is intended for the *ex vivo* activation and expansion of human T lymphocytes without the need for serum supplementation or additional cytokines.

3. Recommended Materials

The following materials are recommended for use with Synecta™ T1:

- CTS™ OpTmizer™ T Cell Expansion SFM with OpTmizer™ T-Cell Expansion Supplement (Gibco, Cat. #A1048501)
  - ♦ **Note**: FBS or HABS is not recommended with this media composition
- GlutaMAX™ (Gibco, Cat. # 35050-061, 1X final concentration)
- (OPTIONAL) Physiologix™ XF SR (Nucleus Biologics, Cat #. 320, 2% final concentration)
- Tissue culture treated flat-bottom plates (6, 24, and 96 well formats)
- Tissue culture treated flasks (T25, T75, T182, and T225 formats)
- G-Rex®24 Well Plate (Wilson Wolf, Cat. #80192M)
- AT-Adapt™ Vented Vial Access Device (Aseptic Technologies, Cat. #ADA010) or sterile needle
- Syringe (1-3 mL recommended)

4. Recommended Expansion Conditions

Vessel	Surface Area	T cells per vessel	Synecta™ T1 Volume per vessel
96 well plate flat bottom	0.33 cm^2	$8.25x10^4$	2.5 µL
G-Rex® 24 well plate	2.0 cm^2	$5.00x10^5$	15 µL
6 well plate flat bottom	10 cm^2	$2.5x10^6$	75 µL
T25 Flask	25 cm^2	$6.25x10^6$	187.5 µL
T75 Flask	75 cm^2	$2.0x10^7$	600 µL

5. General Guidelines

- Use Synecta™ T1 at a ratio of 30 µL per 1 x 10^6 T cells. Protocols may require further optimization depending on the application.
- Store Synecta™ T1 at <-65°C upon receipt. The product may be aliquoted and stored at the same temperature. Avoid repeated freeze-thaw cycles.
- Thaw Synecta™ T1 on ice immediately before use.
- For T cell expansion protocols that involve washing steps (e.g. electroporation) or cultures longer than 7 days, cytokine supplementation with media feeding is required.
- When working with T cell cultures, ensure media is at 37°C.

6. 7-Day Expansion Protocol in 96 Well Culture Plates

1. Prepare fresh or cryopreserved T cells for seeding at 8.25 x 10^5 cells/mL in T cell culture medium.
2. Seed 100 µL of T cells per well in a 96 well flat-bottom plate.

3. Using an AT-Adapt™ (or sterile needle) and syringe, withdraw Synecta™ T1 from the AT-Closed Vial®. Transfer to a sterile vial or tube.
4. Mix Synecta™ T1 immediately before use.
   - ♦ **Note**: Mix by pipetting or vortexing for 5-10 sec.
5. Prepare a Synecta™ T1 master mix by combining 2.5 µL of Synecta™ T1 to 97.5 µL of T cell culture medium.
6. Add 100 µL of Synecta™ T1 master mix per well directly to the T cells.
7. (OPTIONAL) Day 0™ Transduction: Add viral vector directly to the T cells when setting up the culture on Day 0.
8. Incubate 96 well plate at 37°C, 5% CO_2 in a humidified incubator for 3 days.
   - ♦ **Note**: On Day 2, cells might be sampled for activation phenotype testing via flow cytometry.
9. On day 3, transfer T cell culture from 96 well plate to a 24 well plate.
   - ♦ **Note**: Mix T cell culture(s) well by pipetting before transferring to 24 well plate.
10. Add 800 µL of warm T cell culture medium per well directly to the T cells.
11. Incubate 24 well plate at 37°C, 5% CO_2 in a humidified incubator for 2-3 days.
12. After 2-3 days in a 24 well plate, observe T cells under a microscope to assess cell density. Once T cell confluence is > 70%, transfer T cell culture(s) from 24 well plate to a 6 well tissue culture treated plate.
    - ♦ **Note**: Mix T cell culture(s) well by pipetting before transferring to 24 well plate.
13. Add 1.5 mL of warm T cell culture medium per well directly to the T cells.
14. Incubate 6 well plate at 37°C, 5% CO_2 in a humidified incubator until day 7 of expansion protocol.
15. On day 7, harvest and wash cells before use in downstream applications.
    - ♦ **Note**: If necessary, T cells can be seeded into larger vessels (e.g. T75 flasks) to continue expansion.

7. 7-Day Expansion Protocol in 24 Well G-Rex® Plate

1. Prepare fresh or cryopreserved T cells for seeding at 5 x 10^6 cells/mL in T cell culture medium.
2. Seed 100 µL of T cells per well.
3. Using an AT-Adapt™ (or sterile needle) and syringe, withdraw Synecta™ T1 from the AT-Closed Vial®. Transfer to a sterile vial or tube.
4. Mix Synecta™ T1 immediately before use.
   - ♦ **Note**: Mix by pipetting or vortexing for 5-10 sec.
5. Add 15 µL of Synecta™ T1 per well directly to the T cells.
6. (OPTIONAL) Day 0™ Transduction: Add viral vector directly to the T cells when setting up the culture on Day 0.
7. Add warm T cell culture medium to reach a final volume of 1 mL per well.
8. Incubate the plate at 37°C, 5% CO_2 in a humidified incubator for 3 days.
   - ♦ **Note**: On Day 2, cells may be sampled for activation phenotype testing via flow cytometry.
9. On day 3, add 6 mL of warm T cell culture medium per well.
10. Continue incubation at 37°C, 5% CO_2 in a humidified incubator for 4 days.
11. After 4 days, harvest and wash cells before use in downstream applications.

8. 7-Day Expansion Protocol in 6 Well Culture Plates

1. Prepare fresh or cryopreserved T cells for seeding at 0.5 x 10^6 cells/mL in T cell culture medium.
2. Seed 5 mL of T cells per well in a 6 well plate.
3. Using an AT-Adapt™ (or sterile needle) and syringe, withdraw Synecta™ T1 from the AT-Closed Vial®. Transfer to a sterile vial or tube.
4. Mix Synecta™ T1 immediately before use.
   - ♦ **Note**: Mix by pipetting or vortexing for 5-10 sec.
5. Add 75 µL of Synecta™ T1 per well directly to the T cells.
6. (OPTIONAL) Day 0™ Transduction: Add viral vector directly to the T cells when setting up the culture on Day 0.
7. Incubate 6 well plate at 37°C, 5% CO_2 in a humidified incubator for 3 days.
   - ♦ **Note**: On Day 2, cells might be sampled for activation phenotype testing via flow cytometry.
8. On day 3, transfer T cell culture(s) from 6 well plate to a T25 flask.
   - ♦ **Note**: Mix T cell culture(s) well by pipetting before transfer.
9. Add 1.25 mL of warm T cell culture medium per flask directly to the T cells.
10. Incubate T25 flask in horizontal position at 37°C, 5% CO_2 in a humidified incubator for 2-3 days.
11. After 2-3 days in a T25 flask, observe T cells under a microscope to assess cell density. Once T cell confluence is > 70%, transfer T cell culture(s) from T25 to a T75 flask.
    - ♦ **Note**: Mix T cell culture(s) well by pipetting before transfer.

12. Add 13.75 mL of warm T cell culture medium per flask directly to the T cells.
13. Incubate T75 flask in horizontal position at 37°C, 5% CO_2 in a humidified incubator until day 7 of expansion protocol.
14. On day 7, harvest and wash cells before use in downstream applications.
    - ♦ **Note**: If necessary, T cells can be seeded into larger vessels (e.g. T225 flasks) to continue expansion.

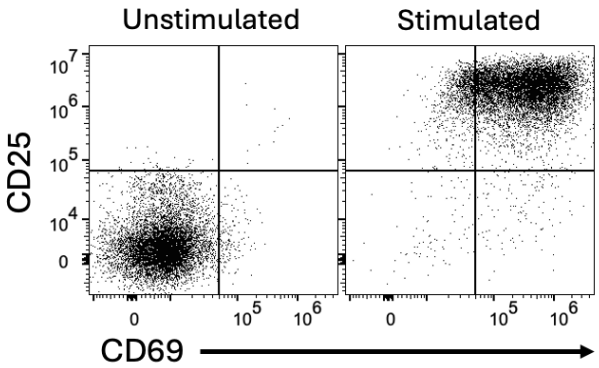
9. 7-Day Expansion Protocol in T25 Flask

1. Prepare fresh or cryopreserved T cells for seeding at 1 x 10^6 cells/mL in T cell culture medium.
2. Seed 6.25 mL of T cells in a T25 flask.
3. Using a AT-Adapt™ (or sterile needle) and syringe, withdraw Synecta™ T1 from the AT-Closed Vial®. Transfer to a sterile vial or tube.
4. Mix Synecta™ T1 immediately before use.
    - ♦ **Note**: Mix by pipetting or vortexing for 5-10 sec.
5. Add 187.5 µL of Synecta™ T1 directly to the T cells.
6. (OPTIONAL) Day 0™ Transduction: Add viral vector directly to the T cells when setting up the culture on Day 0.
7. Incubate T25 flask in horizontal position at 37°C, 5% CO_2 in a humidified incubator for 3 days.
    - ♦ **Note**: On Day 2, cells might be sampled for activation phenotype testing via flow cytometry.
8. On day 3, transfer T cells from T25 flask to a T75 flask.
    - ♦ **Note**: Mix T cell culture(s) well by pipetting before transfer.
9. Add 13.75 mL of warm T cell culture medium per flask directly to the T cells.
10. Incubate T75 flask in horizontal position at 37°C, 5% CO_2 in a humidified incubator for 2-3 days.
11. After 2-3 days in a T75 flask, observe T cells under a microscope to assess cell density. Once T cell confluence is > 70%, transfer T cell culture(s) from T75 to a T182 flask.
    - ♦ **Note**: Mix T cell culture(s) well by pipetting before transfer.
12. Add 25 mL of warm T cell culture medium per flask directly to the T cells.
13. Incubate T182 flask in horizontal position at 37°C, 5% CO_2 in a humidified incubator until day 7 of expansion protocol.
14. On day 7, harvest and wash cells before use in downstream applications.

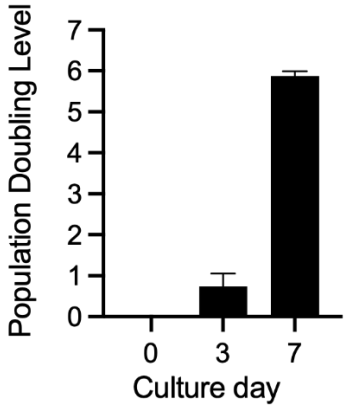
10. 7-Day Expansion Protocol in T75 Flask

1. Prepare fresh or cryopreserved T cells for seeding at 1 x 10^6 cells/mL in T cell culture medium.
2. Seed 20 mL of T cells in a T75 flask.
3. Using a AT-Adapt™ (or sterile needle) and syringe, withdraw Synecta™ T1 from the AT-Closed Vial®. Transfer to a sterile vial or tube.
4. Mix Synecta™ T1 immediately before use.
    - ♦ **Note**: Mix by pipetting or vortexing for 5-10 sec.
5. Add 600 µL of Synecta™ T1 directly to the T cells.
6. (OPTIONAL) Day 0™ Transduction: Add viral vector directly to the T cells when setting up the culture on Day 0.
7. Incubate T75 flask in horizontal position at 37°C, 5% CO_2 in a humidified incubator for 3 days.
    - ♦ **Note**: On Day 2, cells might be sampled for activation phenotype testing via flow cytometry.
8. On day 3, transfer T cells from T75 flask to a T182 flask.
    - ♦ **Note**: Mix T cell culture(s) well by pipetting before transfer.
9. Add 25 mL of warm T cell culture medium per flask directly to the T cells.
10. Incubate T182 flask in horizontal position at 37°C, 5% CO_2 in a humidified incubator for 2-3 days.
11. After 2-3 days in a T182 flask, observe T cells under a microscope to assess cell density. Once T cell confluence is > 70%, split T cell culture(s) from T182 to two T225 flasks.
    - ♦ **Note**: Mix T cell culture(s) well by pipetting before transfer.
12. Add 37.5 mL of warm T cell culture medium per flask directly to the T cells.
13. Incubate T225 flasks in horizontal position at 37°C, 5% CO_2 in a humidified incubator until day 7 of expansion protocol.
14. On day 7, harvest and wash cells before use in downstream applications.

11. Example Data

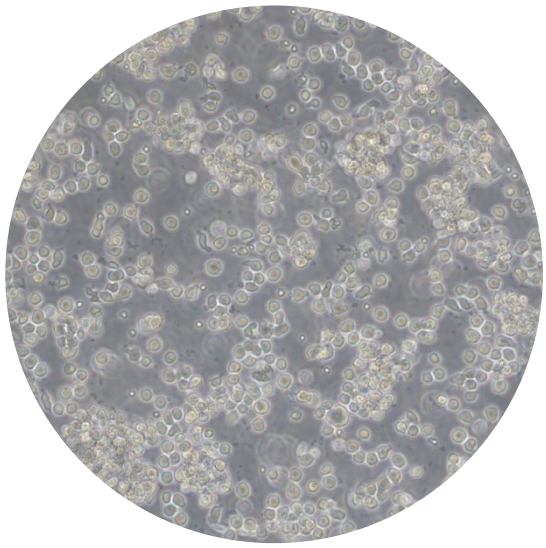


- Isolated human T cells from a healthy donor were activated for 48±4 hours using Synecta™ T1 in CTS™ OpTmizer™ T Cell Expansion media supplemented with OpTmizer™ T-Cell Expansion Supplement and GlutaMAX™ without any cytokine addition.



- Isolated human T cells from a healthy donor were stimulated with Synecta™ T1 at density of 2.5 x 10^5 cells/cm^2 in a 24 Well G-Rex®24 Plate in complete OpTmizer media without any cytokine supplementation. On days 3 and 7, cells were harvested, and T cell population doubling was calculated based on viable cells.

  ♦ **Note**: The activation and fold expansion data shown are representative results from multiple independent experiments and are provided for reference only. Actual results may vary depending on cell donor, culture conditions, and protocol variations.



- Isolated human T cells from a healthy donor were stimulated with Synecta™ T1 at a density of 0.5 x 10^6 cells/mL in a 6 well plate. On day 3, T cells were transferred into a T25 and expanded for another 2 days. Image shows activated T cell morphology at day 5 post-activation.

Dosing Tip

While the protocol includes a recommended Synecta™ T1 dose, using higher doses may further enhance T cell expansion. We invite you to explore different dosing levels to find the conditions that best suit your application.

Technical Support

We are here to support you.

If you encounter challenges or have any questions while using Synecta™ T1, please don't hesitate to contact our team at support@bluewhale.bio. We are happy to work with you to ensure the best results for your experiments.