Synecta™ T1

Catalog No. BWP-010407BE

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Content

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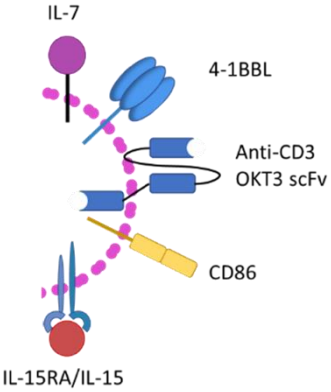
Product	Volume
Synecta™ T1	1 x 1 mL

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 supplementation of serum and optional additional cytokines.

3. Required 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)
- 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)
- G-Rex®24 Well Plate (Wilson Wolf, Cat. #80192M)
- AT-Adapt™ Vented Vial Access Device (Aseptic Technologies, Cat. #ADA010) or disposable needle syringe (1-3 mL recommended)

4. Recommended Expansion Conditions

Vessel	Surface Area	T cells per well	Synecta™ T1 Volume per well
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

5. General Guidelines

- Use of 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 7days, cytokine supplementation/feeding is required.
- When working with T cell cultures ensure media is at 37°C.

6. 7-Day Expansion Protocol in Tissue 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 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. 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 and Synecta™ T1 directly to the T cells.

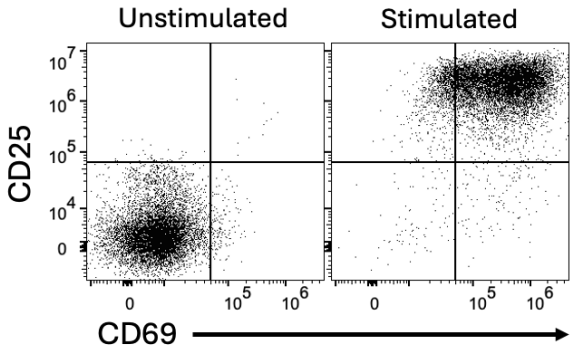
8. Incubate 96 well plate at 37°C, 5% CO_2, humidified incubator for 3 days.
   - **Note**: On Day 2, cells might be sampled for activation phenotype testing via flow cytometry.
9. On day 3, transfer T cells culture from 96 well plate to a 24 well plate.
   - **Note**: Mix T cell culture(s) well by pipetting before transferring to 24 well plate.
10. Add 800 µL of T cell culture medium per well directly to the T cells.
11. Incubate 24 well plate at 37°C, 5% CO_2, 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 density 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 T cell culture medium per well directly to the T cells.
14. Incubate 6 well plate at 37°C, 5% CO_2, 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) for to continue expansion.

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

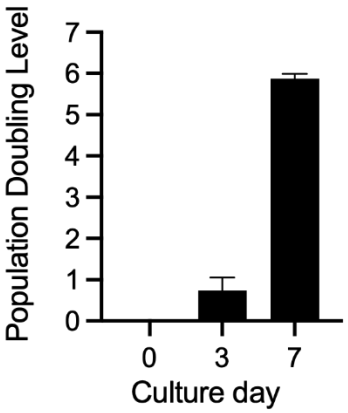
1. Prepare fresh or cryopreserved T cells for seeding at 5 x 10^6 cells/mL in T cell culture medium.
2. Seed 100 µL of T cells per well.
3. Using a AT-Adapt™ (or sterile needle) and syringe, withdraw Synecta™ T1 from the AT-Closed Vial®. Transfer to a sterile vial or tube.
4. Mix Synecta™ T1 immediately before use.
   - **Note**: Mix by pipetting or vortexing for 5-10 sec.
5. Add 15 µL of Synecta™ T1 per well directly to the T cells.
6. (OPTIONAL) Day 0™ Transduction: Add viral vector and Synecta™ T1 directly to the T cells.
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, 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 media per well.
10. Continue incubation at 37°C, 5% CO_2, humidified incubator for 4 days.
11. After 4 days, harvest and wash cells before use in downstream applications.

Example Data

a) Isolated human T cells from a healthy donor were activated for 48±4 hours using Synecta™ T1 in complete OpTmizer media without any cytokine supplementation.



b) 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.

Dosing Tip

While the protocol includes a recommend 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.