

Frequently Asked Questions (FAQ)

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General Questions

What is the Synecta™ platform?

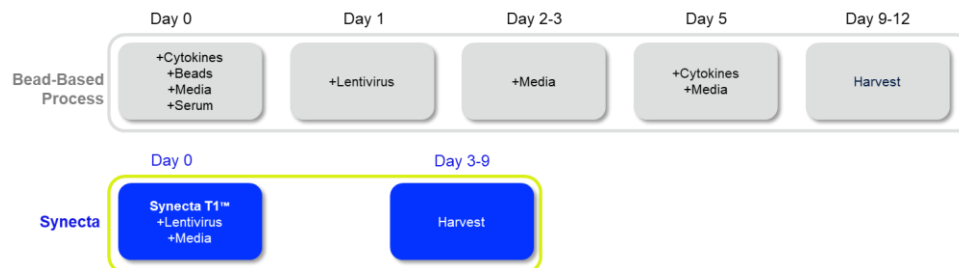
- The Synecta™ platform employs cell-derived nanoparticle (CDNP) technology to deliver activation, expansion, differentiation, or other biological signals to cells in a controlled and scalable manner.

What is Synecta T1 and what are the primary applications of Synecta™ T1?

- Synecta T1 is a CDNP product engineered for T cell activation and expansion. It simplifies and accelerates *ex vivo* T cell workflows across research, preclinical development, clinical translation, and GMP commercial manufacturing.

What does a typical workflow with Synecta T1 look like?

- Synecta T1 can be used directly with isolated T cells or PBMCs
- When using a lentivirus vector, we recommend adding it on Day 0 together with Synecta T1
- For processes with shorter than 9 days of expansion, no soluble cytokines are needed. Please contact our technical support team for alternate protocols and guidance.



What are the shipping and storage conditions for Synecta T1? How long is the shelf life for Synecta T1?

- The product is shipped on dry ice and should be stored at <-65 °C long-term. Once thawed, it may be aliquoted/used once and then refrozen once if necessary. For optimal results, we recommend limiting the product to a single freeze–thaw cycle.
- Synecta T1 is stable for at least 1 year at <-65 °C.

What safety and regulatory considerations apply when using Synecta T1?

- Synecta T1 CDNPs are non-replicating and are used exclusively *ex vivo* during T cell manufacturing. Synecta products are ancillary materials and are not intended for *in vivo* use.

- Synecta T1 is supported by BlueWhale’s FDA-accepted Drug Master File (DMF), which is referenced in a clinical trial at the University of Pennsylvania (NCT04684563). In addition, BlueWhale has received FDA Advanced Manufacturing Technology (AMT) designation for the Synecta CDNP platform, enabling developers to reference an FDA-recognized technology and support efficient progression from research to clinical development and commercialization.

What cell types can Synecta T1 activate?

- Synecta T1 is designed to selectively activate T cells and is compatible with multiple T cell isolation protocols for positively or negatively selected T cells.
- Synecta T1 may be used directly with PBMCs; however, due to the heterogeneous cellular composition of PBMCs, optimization of the working dose is typically required to support consistent activation and expansion. For workflows that rely on predefined or standardized Synecta T1 dosing, the use of purified T cell inputs is generally recommended.
- Synecta T1 has been evaluated with tumor-infiltrating lymphocytes (TILs), NK-T, and gamma-delta T cells; however, dose optimization may be required for these cell types.
- **Best practice:** For first-time use or when working with defined dosing conditions, start with purified T cells. If using other cell type, perform a small-scale dose-response study to identify the optimal Synecta T1 concentration. Our support team is here to support you with ready-made experimental designs and parameters, please contact us at supportf@bluewhale.bio for more information.

Will Synecta T1 GMP product be available?

- GMP-grade Synecta™ T1 is planned through our supply partnership with Avantor.

What level of technical support is available for Synecta T1 users?

- BlueWhale Bio has a dedicated technical solutions team to ensure successful testing, implementation, and use of Synecta. BlueWhale Bio also provides quality and regulatory resources to support customers entering into Commercial Supply Agreements.

Where can I find product documentation, SOPs, and technical resources?

- Documentation, protocols, and technical resources can be found on the BlueWhale Bio website (<https://bluewhale.bio/our-products/>)

How do I contact BlueWhale Bio for ordering, support, or training inquiries?

- Synecta™ T1 is available for purchase by contacting our Sales team at BD@bluewhale.bio or through our website at <https://bluewhale.bio/products/synecta-t1/>. For technical support, training, or any questions related to product use, please contact support@bluewhale.bio

Technical Questions

What is the composition of Synecta™ T1?

- Synecta T1 consists of cell-derived nanoparticles engineered to display defined T cell activation, co-stimulatory ligands, and cytokines. They include anti-CD3 scFv, co-stimulatory signals CD86 and 4-1BBL, and membrane-bound cytokines IL-15/IL-15R α and IL-7, all on a lipid bilayer. This cell-mimetic surface supports T cell activation and expansion by enabling natural engagement of the T cell receptor.

Why are 4-1BBL and IL-15R α part of Synecta T1?

- 4-1BBL (CD137L) provides a co-stimulatory signal that enhances T cell survival, metabolic fitness, and memory formation compared to CD28-only activation. By incorporating membrane-bound 4-1BBL, Synecta™ T1 promotes robust expansion without driving exhaustion. This results in more durable, functionally-fit cells for advanced immunotherapy applications.
- IL-15R α enables physiologic trans-presentation of IL-15, closely mimicking how IL-15 is naturally delivered. When IL-15 is associated with IL-15R α , the cytokine signal is stable, localized, and efficiently delivered to responding T cells.

How are the levels of transmembrane cytokines measured?

- During production of Synecta T1, expression of transmembrane cytokines and ligands is monitored by flow cytometry during stable cell line generation. Cytokine levels in the final product are measured using ELISA-based assays.

Protocol-Related Questions

What are important process considerations when using Synecta™ T1?

- During the activation phase (0–48 hours), maintaining appropriate cell-to-CDNP proximity is important for effective signaling. Over-diluted cultures may show reduced activation and expansion. Static culture is recommended during this period, as agitation can disrupt cell-CDNP interactions. Refer to the Synecta T1 protocol for recommended cell-density and volume ranges.
- If centrifugation or wash steps are introduced during the expansion phase, additional cytokine supplementation is recommended since membrane-associated cytokines are removed with the supernatant. This is particularly relevant for workflows involving gene editing or gene delivery, such as electroporation.
- Early sampling should be done judiciously. Excessive sampling in the first few days can disrupt the intended cell-to-particle ratio, as the particles also serve as a cytokine source, which may negatively impact expansion. If early readouts are required, we recommend including an additional sample specifically designated for testing.
- For cultures extending beyond seven days, supplemental cytokines should be added to support continued cell growth and viability, as membrane-bound cytokines are progressively depleted over time. Please contact support@bluewhale.bio for additional information

Is Synecta T1 compatible with different culture media?

- Yes. Synecta T1 is compatible with a range of commonly used T cell culture media, including
 - i) OpTmizer™ + 1% GlutaMAX™
 - ii) NB-ROC + 2% Physiologix™
 - iii) X-VIVO™ 15 + 5% hABs + 1% GlutaMAX™
 - iv) RPMI + 10% FBS + 1% HEPES + 1% P/S
 - v) T-Vivo™ + 1% GlutaMAX™

Optional serum replacements (can further support cell growth)

- i) 2% Physiologix™
- ii) 2.5% Thermo ICSR™

Certain media compositions are not compatible with the use of serum. For guidance on alternative media compatibility, please contact support@bluewhale.bio

Can Synecta™ T1 be adapted for custom workflows or protocols?

- Yes. Synecta T1 is designed to be workflow-agnostic and can be integrated into a wide range of T cell manufacturing protocols. The platform has been evaluated across different media systems, culture formats, including automated bioreactors, and processing timelines, and can be adapted to meet specific research or translational needs.
- Our Technical Solutions team can assist in planning your study design to ensure optimal performance and successful integration. Please support@bluewhale.bio

How does Synecta T1 fit into viral transduction or virus-free workflows?

- Synecta T1 is compatible with both viral and virus-free cell engineering approaches. Viral transduction is commonly performed on Day 0 following activation; however, in some workflows, Day 1 transduction has shown comparable outcomes. Synecta™ T1 has also been used in virus-free genome editing strategies, providing flexibility in the choice and timing of engineering approaches. Performance may vary depending on cell source, vector system, and overall workflow configuration.
- Our Technical Solutions team can assist in planning your study design to ensure optimal performance and successful integration. Please support@bluewhale.bio

Is Synecta T1 compatible with automated or closed-system workflows?

- Yes. Synecta T1 is compatible with automated and closed-system platforms, including static culture formats and bioreactor-based systems. The bead-free, biodegradable format supports integration into workflows that aim to minimize open handling and manual intervention.
- Synecta T1 has been evaluated in multiple automated and closed-system platforms. Please contact customer support to inquire about data with your preferred system.

Does Synecta T1 require IL-2 supplementation?

- IL-2 supplementation is generally not necessary as Synecta T1 particles provide embedded cytokine support. When IL-2 is included, standard low-dose ranges are generally sufficient. Optimal cytokine supplementation strategies may vary based on cell source, culture media, and the desired cellular phenotype.

How can residual CDNPs be removed from T cell cultures?

- Synecta is a biodegradable platform and does not require any removal steps.

- Synecta can be easily removed by centrifugation and washing if a protocol calls for steps early in the activation while Synecta is still present.