

Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP

Catalog# / Size	420502 / 50 mL
Other Names	Xeno-free, Serum-free Human Serum Replacement
Description	<p>Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP is specifically formulated to support PBMC-derived T and NK cell expansion, resulting in equal or higher specific cell numbers compared to the use of human AB serum. It is a xeno-free, serum-free formulation, prepared without bovine or other animal components, intended to be combined with the basal media of choice, such as IMDM. It is suitable for use in combination with several T cell activation methods and is able to support T cell populations (CD4, CD8) comparable to human AB serum. When used under the appropriate conditions, this product can result in increased naïve T cell numbers. This GMP product is suggested for use in research and further manufacturing. The benefits of this serum substitute include:</p> <ul style="list-style-type: none">•Effective for generation of naïve CD3+ T cells from culture.•Effective for maintaining CD4/CD8 balance.•Maintains high cell viability, >90%, throughout cell culture.•Effective for NK cell expansion.•Minimizes safety risks associated with human AB serum.•Improved lot-to-lot consistency over human AB serum.•Saves time and costs required to qualify human AB serum

Product Details

Formulation	Xeno-free, serum-free formulation
Storage & Handling	Store at -20°C to -5°C. Thaw overnight at 4°C. If necessary product can be kept at 4°C up to two weeks.
Application	Cell Culture
Recommended Usage	Recommended use at 5% v/v in basal media, combined with 200 IU/mL of human recombinant IL-2. Product should be titrated (2.5-10%) to determine optimal performance for your culture conditions.
Application Notes	Note: This product contains human-derived components.

Complete cell Culture Media Preparation:

Thaw Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP overnight at 4°C. The product can be stored at 4°C in the dark up to 2 weeks post-thaw. **Do not refreeze after initial thaw.** Presence of cloudiness in the product after thaw is normal, and relates to the high enrichment of the product. Make sure to mix product prior to use. Once the product is diluted into basal media, no cloudiness should be observed.

Dilute Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP to 5% v/v in IMDM and 200 IU/mL of recombinant human IL-2 (BioLegend, Cat. No. 791906 or equivalent). Alternatively, the product can be used in combination with your basal media of choice and cytokines of interest. To determine optimal performance, a titration of the product under your desired cell culture setup may be required.

Cell Culture Platform:

Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP can be used with tissue culture plates, flasks and the G-REX® system. If using a bag format, testing is recommended with the specific bag being used.

Cell Activation methods:

Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP can be used in combination with anti-human CD3 and CD28 antibodies in solution, plate-coated or conjugated to magnetic beads. Optimal values should be determined if using a different culture setup.

For the protocols below, complete media refers to IMDM with 5% v/v Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP and 200 IU/mL recombinant human IL-2 (BioLegend, Cat. No. 791906 or equivalent) for T cell culture, and 1000 IU/mL for NK cell culture.

1. Coating plates with anti-human CD3 and CD28 antibodies

Add 1 µg/mL of anti-human CD3 (OKT3 clone, BioLegend, Cat. No. 317326 or equivalent) and 1 µg/mL of anti-CD28 antibodies (CD28.2 clone, BioLegend, Cat. No. 302934 or equivalent) to PBS. Add solution to the vessel, at recommended volume (table below). Incubate for 2 hrs at 37°C in a tissue culture incubator (or overnight at 4°C, preventing evaporation with Parafilm®). Aspirate solution and wash twice with 1 volume of PBS. Wells are ready to be used. Do not let wells dry.

TABLE 1: Recommended volumes of coating solution composed of anti-human CD3 and CD28 antibodies.

Vessel	Recommended volume
12- well plate	0.75 mL
6- well plate	2 mL
T25 flask	5 mL
T75 flask	15 mL

2. Soluble antibodies

Add 10 µg/mL of anti-human CD3 (OKT3 clone, BioLegend, Cat. No. 317326 or equivalent) and 5 µg/mL of anti-CD28 antibodies (CD28.2 clone, BioLegend, Cat. No. 302934 or equivalent) to complete media.

PBMC-Derived T cell culture protocol:

1. Equilibrate appropriate amount of complete media at 37°C for 10-15 min.
2. Plate PBMC (fresh or frozen) at 1×10^6 cells/mL and incubate at 37°C and 5% CO₂.
3. Allow cells to get activated for 3-4 days.
4. At day 3-4, dissociate cell clumps by pipetting until a single cell solution is observed.
5. Determine cell density.
6. Dilute cell culture to 0.25 - 0.5 x 10⁶ cells/mL and incubate at 37°C and 5% CO₂.
7. Repeat steps 4-6 every 2 days.
8. Monitor cell culture to make sure confluency is not reached. Pending donor-to-donor variability exponential expansion may require more frequent feeding. Complete media has been shown to support up to 4 x 10⁶ cells/mL without impact on cell viability and quality.
9. Follow with further processing and/or cell analysis.

PBMC-Derived NK cell culture protocol using K562 feeder cells:

1. Start by inactivating K562 cells with mitomycin-C (20 µg/mL in K562 growth media) for 45 min at 37°C and 5% CO₂.
2. Spin down inactivated K562 cells at 300xg for 5 min. Wash cell pellet twice with fresh media, to remove any leftover mitomycin-C.
3. Equilibrate appropriate amount of complete media at 37°C for 10-15 min.
4. Plate PBMC (fresh or frozen) at 0.5 x 10⁶ cells/mL and incubate at 37°C and 5% CO₂.
5. Add feeder cells (inactivated K562 cells (recommended value of 1 K562 cell to 2 PBMC cells; optimal value can be determined for desired culture setup).
6. Allow NK cells to get activated for 2-3 days.
7. Determine cell density.
8. Dilute cell culture to 0.25 - 0.5 x 10⁶ cells/mL and incubate at 37°C and 5% CO₂.
9. Repeat steps 4-6 every 2 days.
10. Re-feed NK cell culture with freshly inactivated K562 cells at day 7.
11. Repeat steps 4-6 every 2 days up to day 14 (or appropriate culture end).
12. Follow with further processing and/or cell analysis.

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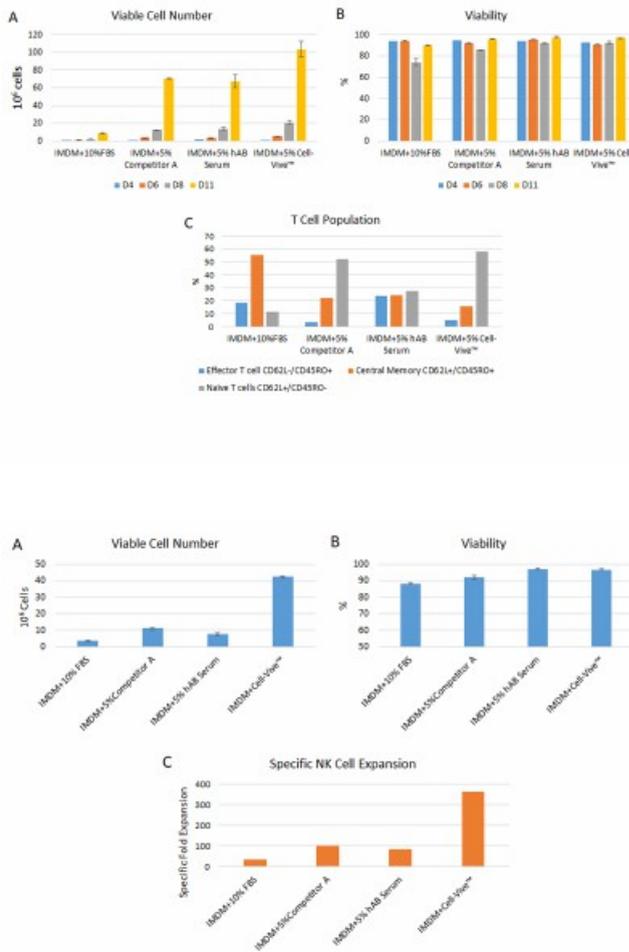
GMP Serum Substitute. BioLegend products are manufactured in an ISO 13485:2016-certified facility following GMP compliant procedures to ensure the highest quality standards. For research or further manufacturing use. Not for use in diagnostic or therapeutic procedures. Our processes include:

- Batch-to-batch consistency
- Material traceability
- Documented procedures
- Documented employee training

- Equipment maintenance and monitoring records
- Lot-specific certificates of analysis
- Quality audits per ISO 13485:2016
- QA review of released products

BioLegend GMP Serum Substitute products are manufactured and tested in accordance with USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and Ph. Eur. Chapter 5.2.12.

Product Data



Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP can lead to higher PBMC derived T cell expansion, in particular of Naïve T Cells. PBMC-derived T cells were activated with 1 µg/mL of Ultra-LEAF™ anti-human CD3 antibody (Cat. No. 317347), 1 µg/mL of Ultra-LEAF™ anti-human CD28 antibody (Cat. No. 302943) in IMDM basal media supplemented with 200 IU/mL Recombinant human IL-2 (791908) and appropriate supplements. Cell number (A) and viability (B) were determined at day 4, 6, 8 and end of culture (day 11). Flow cytometry was used to determine specific T cell populations, by making use of CD62L and CD45RO cell markers (C).

Cell-Vive™ T-NK Xeno-Free Serum Substitute, GMP can lead to higher PBMC derived NK cell expansion. PBMC-derived T cells were cultured in IMDM basal media supplemented with 1000 IU/mL Recombinant human IL-2 (791908) and appropriate supplements, in the presence of inactivated K562 feeder cells at ratio of (2:1). Cell number (A) and viability (B) were determined at day 14. C) Specific NK cell expansion was determined by combining total viable cell number generated with the % of NK cells. NK cell population was identified by flow cytometry as being CD3- and CD56+ cells.

Symbols Glossary*

Symbol	Meaning	Symbol Title	Symbol No.	Symbol	Meaning	Symbol Title	Symbol No.
	Catalog number	Catalogue number	5.1.6		Indicates the need for the user to consult the instructions for use.	Consult instructions for use	5.4.3
	Indicates the temperature limits to which the medical device can be safely exposed.	Temperature limit	5.3.7		Indicates a medical device that needs protection from light sources.	Keep away from sunlight	5.3.2
	Indicates the upper limit of temperature to which the medical device can be safely exposed.	Upper limit of temperature	5.3.6		Indicates the date after which the medical device is not to be used.	Use-by date	5.1.4
	Indicates the medical device manufacturer.	Manufacturer	5.1.1		Indicates the authorized representative in the European Community.	Authorized representative in the European Community	5.1.2
	Indicates the manufacturer's batch code so that the batch or lot can be identified.	Batch code	5.1.5		Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.	In vitro diagnostic medical device	5.5.1

* Symbol information is from EN ISO 15223-1:2016 Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied – Part 1: General requirements

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