

Product Information

Medium B
for Cultivation of T, NK, and CD34⁺ Cells, with L-Glutamine
sterile-filtered
Cat. No. FT-25-093-100ML (100 ml)

General Information

Medium B is a broad-range culture medium designed for the cultivation of peripheral blood mononuclear cells (PBMCs) and selected immune cell types derived from human blood, including T cells, NK cells and CD34⁺ progenitor cells. It provides high performance directly after primary cell isolation and supports robust and reproducible growth of PBMC-derived cell lines. Reliable performance is maintained even when working with variable or low-quality blood samples.

Medium B was developed and validated for the culture and expansion of various different immune cell types including T cells, defined T cell subsets (CD4⁺, CD8⁺ and CAR T cells), NK cells, CD34⁺ cells CIK cells and various immune cell lines.

The formulation does not contain interleukins, allowing full flexibility for customized activation and differentiation protocols. Medium B is defined and can be used with or without serum. When serum is applied, a final concentration of 2-5% FBS is recommended.

Product Specifications

Appearance	Clear red liquid
Specifications	Xeno-free, serum-free with Phenol Red with L-Glutamine w/o Cytokines
Storage and shelf life	Store at +2°C to +8°C Protect from light!
Shipping conditions	Cool packs

Instructions for Use

Important Notes for Medium Qualification

Medium B is suitable for serum-free cultivation of various cell types. However, for certain applications supplementation with 2-5% FBS might be required. **Therefore, a parallel testing strategy with Medium B without FBS and Medium B with 2% FBS is strongly recommended.**

General Notes

- For T cell cultivation, liquid height should not exceed 1.0 – 1.2 cm when cultured in plates and 0.3 cm when cultured in flasks.
- For T cell cultivation, a shaking rate of 8 rpm at 6° is recommended.
- For NK cell cultivation, Medium B can be used with or without the use of feeder cells.
- Medium B is suitable for standard transduction protocols. Please note that due to the serum-free composition of Medium B, transduction can be delayed for 24 to 48 hours.
- For nuclear- or electroporation, the incubation time between activation and transduction should be prolonged for 24 to 48 hours in contrast to serum-containing media.
- After adding cytokines to the medium, use within one week. Fresh supplementation is recommended.

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Cultivation Overview for Different Cell Types

Medium B is suitable for different cell types. The table below provides an overview of typical seeding and expansion cell densities. Cell density can be optimized according to specific protocols and aims.

Cell Type	Seeding Density
Primary T Cells	For activation 0.5×10^6 - 1.0×10^6 cells/ml During expansion 0.5×10^6 - 1.0×10^6 cells/ml
CAR T Cells	Before transfection: 1×10^6 - 1.5×10^6 cells/ml Post-transfection: 5×10^5 - 1×10^6 cells/ml
CIK Cells	For activation: 1×10^6 - 2×10^6 cells/ml During expansion: 1×10^6 - 2×10^6 cells/ml
NK Cells	For seeding and expansion 5×10^5 - 1×10^6 cells/ml

CD3/CD28 Plate Coating (if required)

T cells can be activated using either CD3/CD28 antibody-coated plates or activation beads. When using antibody-coated plates, PBMCs should remain in the coated wells only during the initial 3 days of culture. Perform the coating procedure before isolating PBMCs:

1. Prepare a sterile coating solution by diluting anti-CD3 and anti-CD28 antibodies to a final concentration of 1 µg/ml each in cell culture grade water (Cat. No. WAT-500ML) or water for injection grade water (Cat. No. WFI-500ML).
2. Fill the outer wells of the plate with PBS (Cat. No. PBS-1A) to minimize evaporation.
3. Add the appropriate volume of coating solution to the desired wells and seal the plate with Parafilm. Incubate overnight at +2°C to +8°C or for 2 hours at 37°C.
4. After incubation, carefully remove the coating solution.
5. Wash the wells once with PBS without allowing the surface to dry.
6. Remove the PBS completely before seeding the cells.
7. Immediately seed PBMCs at the appropriate cell concentration in supplemented Medium B into the coated wells.

PBMC Isolation and Cell Thawing

Isolate PBMCs from whole blood using Lymphocyte Separation Medium (LSM) (Cat. No. LSM-A) according to the manufacturer's instructions. Frozen cells can be thawed according to standard protocols in a 37°C water bath.

T Cell Expansion in Well Plates or Shaker Flasks

1. After isolation, transfer cells to Medium B supplemented with cytokines appropriate for the specific cell type, for example IL-7 (20 ng/ml) and IL-15 (20 ng/ml).
2. Adjust the cell concentration to $0.5 - 1.0 \times 10^6$ cells/ml and seed them in CD3/CD28-coated plates.
Note: within the first two days after seeding, lentiviruses or retroviruses carrying the target gene can be added to the culture for transduction.
3. Incubate the cells for three days in a humidified 5% CO₂ incubator at 37°C.

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4. After the initial 3-day activation period, gently resuspend the cells and transfer them to uncoated culture vessels. Examine under the microscope for cell morphology and count viable cells.
5. Maintain the following parameters during passaging:

Inoculation Density	0.5 – 1.0 × 10 ⁶ cells/ml
Medium & Supplementation	Medium B + cytokines according to cell type and protocol (e.g., 20 ng/ml IL-7, 20 ng/ml IL-15) Optional: + 2-5% FBS
Culture Conditions	5% CO ₂ , 37°C, humidified incubator Check cell growth each two days: If cells are dense, expand to a larger culture flask or split cells. If cell density is low, refresh the medium on day 2 and wait one more day before expansion.

Long-term culture:

- T cells can be maintained for up to 17 days.
- For prolonged expansion, re-stimulate cells on CD3/CD28-coated plates approximately after 14 days.
- Do not exceed 4 days without medium exchange, as growth rate declines.

T Cell Expansion in G-Rex Perfusion Systems

6. Transfer cells in Medium B supplemented with cytokines appropriate for the specific cell type, for example IL-7 (20 ng/mL) and IL-15 (20 ng/mL).
7. Adjust the cell concentration to 0.5 – 1.0 × 10⁶ cells/cm² and add appropriate T cell activation factors such as CD3 and CD28 antibodies or CD3/CD28 coated beads.
8. Incubate the cells in a humidified 5% CO₂ incubator at 37°C.
9. After two to three days, freshly supplemented Medium B can be added directly up to the maximum working volume.
10. Maintain the following parameters:

Medium & Supplementation	Medium B + cytokines according to cell type and protocol (e.g., 20 ng/ml IL-7, 20 ng/ml IL-15) Optional: + 2% FBS
Culture Conditions	5% CO ₂ , 37 °C, humidified incubator Check cell growth each day: Add new cytokines each two days Replace 75% of Medium B each four days

Note: Sample cells every 24 to 48 hours to determine VCD and cell density. Maximum recommended cell density is 2.0 – 4.0 × 10⁷ cells/cm².

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Precautions and Disclaimer

This product is for research use and further manufacturing only.

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).