HemEx™-Type9AØ

Instruction for use

CELL SCIENCE & TECHNOLOGY INSTITUTE INC.

ver.2

Product Description

HemEx™-Type9AØ is a basal medium for expansion of mouse hematopoietic stem cells (HSCs). The culture method of HSCs from bone marrow reported by Becker et al. (Ref. 1) enable expansion of HSCs from single bone marrow-derived (BM) HSC with albumin-free, chemically-defined medium based on Ham's F-12 Nutrient Mixture. HemEx™-Type9AØ simplify to prepare the culture medium used in this culture method. This product contains only Insulin-Transferrin-Selenium-Ethanolamine (ITS-X) as supplement, which allows users flexible combination of cytokines and small molecules.

Product	Catalog Number (NIPRO/CSTI)	Volume	Storage	Shelf life
HemEx™-Type9AØ	87-590 / A5P10P01C	100 mL	2-8 ℃ ; Protect from light	N/A[1]

[1] Product shelf life is under testing.

Formulation

This product contains

- Ham's F-12 Nutrient Mixture
- 0.01% Soluplus®
- 10 mM HEPES
- 1 mM L-Glutamine
- 1× ITS-X

This product does NOT contain antibiotics and other cytokines.

Preparation of culture medium

Add antibiotics such as Penicillin-Streptomycin (final conc. 1x) if necessary.

For the complete medium, it requires addition of the following compounds by stock solution.

- Thrombopoietin at a final conc. of 100 ng/mL
- SCF at a final conc. of 10 ng/mL

Prepare complete media just before use. Media should be pre-warmed to 37 ˚C before use.

Prepare stock solutions shown in the following table.

Cytokines	Final conc.	Stock solution
Mouse TPO Recombinant Protein (Gibco # 315-14)	100 ng/mL	100 µg/mL in F-12 medium
Mouse SCF Recombinant Protein (Gibco #250-03)	10 ng/mL	10 µg/mL in F-12 medium

Cell Culture protocol of HSCs

1. Prepare murine HSCs.

Whole bone marrow cells are collected from murine pelvic, femur and tibia bones. The obtained cells are then sorted by FACS to purify HSCs. CD201+CD150+KL cells from C.B-17 SCID mouse or CD34-CD150+KSL cells from C57BL/6 mouse are seeded as HSCs.

<Bulk culture>

2. Suspend cells with the complete medium at the density of 5 x 10^3 cells/mL.
3. Seed 1 mL of cell suspension to human fibronectin-coated 24-well dishes (Corning # 354411).
4. Incubate at 37 ˚C with 5% CO_2 in humidified incubator.
5. Change culture media on day 3 after seeding. Then maintain cell culture by changing media every 2-3 days throughout the entire culture.

6. For change media, gradually remove media from near the meniscus to avoid disturbing cells. Aim to remove ~95% of the medium.

<Single cell culture>

2. Sort single HSCs into individual wells on a 96-well U-bottom plate (TPP #92697) prefilled with 200 mL of the complete medium.
3. Incubate at 37 ˚C with 5% CO_2 in humidified incubator.
4. Change culture media on day 7 post-sort. Then maintain cell culture by changing media every 2-3 days throughout the entire culture.

[Note]

- HSCs can expand 9-fold in number after 10 days in culture. Cell cultures can be analyzed at any time point by flow cytometry.
- Gene editing can be applied after 3 days of bulk culture. Combined with single cell culture, desired HSC clone can be screened and transplanted. Please refer to Ref. 1 for detail.
- Please refer to Ref. 1 and Ref. 2 for detailed protocol of HSCs culture. The information of cell culture supplements, cell analysis reagents, supplies and equipment is also mentioned in Ref. 1 and Ref. 2

Intended use

This product is for research use only. Not for therapeutic or diagnostic use.

Reference

1 Becker HJ, Ishida R, Wilkinson AC, Kimura T, Lee MSJ, Coban C, Ota Y, Tanaka Y, Roskamp M, Sano T, Tojo A, Kent DG, Yamazaki S. Controlling genetic heterogeneity in gene-edited hematopoietic stem cells by single-cell expansion. Cell Stem Cell. 2023 Jul 6;30(7):987-1000.e8. doi: 10.1016/j.stem.2023.06.002. Epub 2023 Jun 28. PMID: 37385251; PMCID: PMC10338855.

2 Wilkinson AC, Ishida R, Nakauchi H, Yamazaki S. Long-term ex vivo expansion of mouse hematopoietic stem cells. Nat Protoc. 2020 Feb;15(2):628-648. doi: 10.1038/s41596-019-0263-2. Epub 2020 Jan 8. PMID: 31915389; PMCID: PMC7206416.

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