HemEx™-TypeH

Instruction for use

CELL SCIENCE & TECHNOLOGY INSTITUTE INC.

ver.3

Product Description

HemEx™-TypeH is a basal medium for expansion of human hematopoietic stem cells (hHSCs). The culture method of hHSCs from umbilical cord reported by Sakurai et al. (Ref. 1) enable expansion of LT-HSCs from umbilical cord blood-derived (CB) hHSCs with albumin-free, chemically-defined medium based on Iscove's MDM. Modified medium reported by Ishitsuka (Ref. 3) made possible expansion of peripheral blood stem cells (PBSCs). HemEx™-TypeH simplify to prepare the culture medium used in this modified culture method. This product contains only Insulin-Transferrin-Selenium-Ethanolamine (ITS-X) as supplement, which allows users flexible combination of cytokines and small molecules such as UM729.

Product	Catalog Number (NIPRO/CSTI)	Volume	Storage	Shelf life
HemEx™-TypeH	87-079 / A2D10P01C	100 mL	2-8 ℃ ; Protect from light	N/A[1]
Related Product	**Catalog Number (NIPRO/CSTI)**	**Volume**	**Storage**	**Shelf life**
200 mM L-Glutamine solution	87-885/A5300P01C	100 mL	-20 ℃ ; Protect from light	15 months

[1] Product shelf life is under testing.

Formulation

This product contains

- Iscove's Modified Dulbecco's Medium
- 0.01% BASF Solplus®
- 1× ITS-X

This product does NOT contain L-glutamine, antibiotics, other cytokines, activators and inhibitors.

Preparation of culture media

Add L-glutamine (final conc. 4 mM) before use. Add antibiotics such as Penicillin-Streptomycin (final conc. 1x) if necessary.

For complete media (Modified-3a medium in Ref. 3), it requires addition of the following compounds by stock solution.

- Butyzamide at a final conc. of 0.2 μM
- 740Y-P at a final conc. of 5 μM
- UM729 at a final conc. of 1 μM
- Flt3L 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.

Compounds	Final conc.	Stock solution
Butyzamide (Shionogi & Co., Ltd.)	0.2 μM	10 μM in DMSO
740Y-P (CAS No. 1236188-16-1; synthesized)	5 μM	0.5 mM in H2O
UM729 (STEMCELL technologies #72332)	1 μM	1 mM in DMSO
Flt3L (Peprotech #AF-300-19)	10 ng/mL	10 μg/mL in PBS

Cell Culture protocol of hHSCs

1. Prepare hHSCs.

CB hHSCs: purchase CD34+ cells from supplier. Purify CD34+ CD38- cells by FACS after thawing.

PBSCs: Obtain fresh PBSCs from healthy donors. Separate mononuclear cells from total cells after leukapheresis. Enrich CD34+ cells by microbeads kit.

2. Suspend cells with complete media at the density of 2 x 10^4 - 1 x 10^5 cells/mL.

3. Seed 1 mL of cell suspension to 24-well cell culture treated plate.

4. Incubate at 37 ˚C with 5% CO_2 in humidified incubator.

5. Maintain cell culture by changing media every 2-3 days throughout the entire culture.

6. For change media, collect all cell culture media in the well. Centrifuge at 440g for 5 min. Discard supernatant. Re-suspend the cell pellet in fresh complete media at the cell density above. Seed 1mL /well.

[Note]

- CD34+ cells can expand 35-40-fold in number after 21 days in culture. Cell cultures can be analyzed at any time point by flow cytometry. Alternatively, cell cultures can be used in in vivo transplantation assays.
- For purging multiple myeloma cells in PBSCs culture, add 2 µmol/L bortezomib, 1 µmol/L lenalidomide, and 100 ng/mL human recombinant TRAIL in complete media.
- Please refer to Ref. 2 and Ref. 3 for detailed protocol of HSCs culture. The information of cell culture supplements, cell analysis reagents, supplies and equipment is also mentioned in Ref. 2 and Ref.3.

Intended use

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

Reference

1 Sakurai M, Ishitsuka K, Ito R, Wilkinson AC, Kimura T, Mizutani E, Nishikii H, Sudo K, Becker HJ, Takemoto H, Sano T, Kataoka K, Takahashi S, Nakamura Y, Kent DG, Iwama A, Chiba S, Okamoto S, Nakauchi H, Yamazaki S. Chemically defined cytokine-free expansion of human haematopoietic stem cells. Nature. 2023 Mar;615(7950):127-133. doi: 10.1038/s41586-023-05739-9. Epub 2023 Feb 22. PMID: 36813966.

2 Sakurai, M, Ishitsuka, K and Yamazaki, S. "Chemically defined cytokine-free expansion of human haematopoietic stem cells" Protocol Exchange (2023) DOI: 10.21203/rs.3.pex-2163/v1

3 Ishitsuka K, Nishikii H, Kimura T, Sugiyama-Finnis A, Yamazaki S. Purging myeloma cell contaminants and simultaneous expansion of peripheral blood-mobilized stem cells. Exp Hematol. 2024 Mar;131:104138. doi:10.1016/j.exphem.2023.104138. Epub 2023 Dec 25. PMID: 38151170.

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