



YCCEH Protocols

EXPANSION AND DIFFERENTIATION OF HUMAN ERYTHROID POPULATIONS FROM CD34+ CELLS

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Reagents

Expansion media:

- IMDM (base)
- FBS (20% volume)
- SCF(10ng/ml)
- EPO (1U/ml)
- L-Glutamine (1ng/ml)
- Penicillin/Streptomycin (1% volume)
- Beta-mercaptoethanol (75microM)
- IL-3 (1ng/ml)
- Dexamethasone (1 microM)
- β -estradiol 1 microM

Differentiation media:

- IMDM (base)
- FBS (20% volume)
- EPO (1U/ml)
- Penicillin/Streptomycin (1% volume)
- Insulin (1 mg/ml)

Overview

This protocol uses CD34+ cells obtained from YCCEH Cell Prep and Analysis Core (typically available as vials each with 1 million cells) to generate erythroid cells in vitro. Cells are isolated from mobilized peripheral blood mononuclear cells (PBMC) from normal donors. Other sources of starting cells including CD34+ cells (from bone marrow or cord blood) can be used. Unfractionated peripheral blood, bone marrow or mobilized PBMC are also used in some protocols.

Procedure

Thawing Cells:

Filtered PBS with 1% FBS is used to thaw CD34+ vials. It should be at room temperature.

Quickly thaw the vial in 37°C water bath. This should take no more than 3-5 minutes. Gently shake cells during the thaw.

Add the vial to 1 ml of PBS-FBS in a 50 cc conical tube.

Swirl for about 1 minute to equilibrate. Then add equal volume (2 ml) of PBS-FBS.

Add double the volume of 1% FBS (4, 8 and 16 ml)

Continue till the volume is 32 ml.

Spin cells for 5 minutes at 180g force. Remove supernatant carefully and proceed to resuspension in expansion media.

Culture Expansion of the CD34 cells

Resuspend cells in expansion media ($1-2 \times 10^5$ / ml) and culture in 37 degrees, 5% CO₂. Monitor cell numbers each day and ensure that cell concentration does not exceed 0.5×10^5 in the initial 4-5 days of culture.

Following this time , up to 1×10^6 / ml is acceptable.

Expansion can be done for 10-14 days prior to differentiation to terminal phase.

Following expansion phase, move to differentiation media. This can last for 4-5 days.

Notes:

1. Batch to batch variation is expected with primary cultures.
2. FACS analysis every 3-4 days for erythroid markers (CD71 and CD235A) is recommended to ensure timely maturation.
3. GCSF-mobilized unfractionated cells can be variable in their monocyte/macrophage content. High monocyte content will result in high fraction of macrophages in culture which will inhibit erythropoiesis. For that reason, this source is not recommended, although they likely have enough progenitors for
4. > 95% of cells is expected to be erythroid when starting with CD34+ cells. A variable proportion of non-erythroid cells will remain in culture which will likely not be problematic for most studies.
5. FBS can be eliminated with minimal change in results (fewer cells in expansion).
6. Cells may suddenly lose viability towards the end of culture period – hence careful monitoring of health of cultures is recommended