This protocol uses CD34+ cells obtained from YCCEH Cell Prep and Analysis Core (typically available as vials each with 1 million cells). 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.

**Thawing:**
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.
- Add the vial to 1 ml of PBS-FBS in a 50 cc conical.
- Swirl for about 1 minute to equilibrate. Then add equal volume (2 ml) of PBS-FBS.
- Continue till the volume is 32 ml.
- Spin cells for 5 minutes at 1200 rpm. Remove supernatant and proceed to resuspension in expansion media.

**Expansion of the CD34 cells**
Resuspend cells in expansion media (1-2 X 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 X 10^5 in the initial 4-5 days of culture. Following that, up to 1 X 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.

**Media:**

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

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

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. > 95% of cells is expected to be erythroid when starting with CD34+ cells. A variable proportion of non-erythroid cells will remain in culture when unfractionated cells are used.
4. FBS can be eliminated with minimal change in results (fewer cells in expansion).
5. Cells may suddenly lose viability towards the end of culture period – hence careful monitoring of health of cultures is recommended.