ISOLATION OF MONONUCLEAR CELLS (PBMC) BY GRADIENT CENTRIFUGATION

Not known

Abstract

Protocol with Ficoll-Paque/Histopaque for isolation of PBMCs from heparinized blood

Citation: Not known ISOLATION OF MONONUCLEAR CELLS (PBMC) BY GRADIENT CENTRIFUGATION. **protocols.io** dx.doi.org/10.17504/protocols.io.rqmd5u6
Published: 16 Jul 2018

Materials

- ✓ Histopaque® or Ficoll-Paque by Contributed by users
- ✓ Heparinized blood by Contributed by users
- ✓ Heat-inactivated fetal bovine serum by Contributed by users
- ✓ RPMI-1640 medium supplemented with 10% FBS by Contributed by users
- ✓ PBS by Contributed by users
- ✓ 50-ml conical tubes by Contributed by users
- ✓ 0.4% Trypan blue solution by Contributed by users

Protocol

Place fresh heparinized blood into 50 ml conical centrifuge tube. Using a sterile pipet, add an equal volume of room-temperature 1 × PBS. Mix well and gently;

Step 1.

In a 50 ml conical tube, place the PBS diluted blood under 10 mL\ Histopaque or Ficoll-Paque PLUS

carefully. *It is important that two layers should be maintained!

Step 2.

Centrifuge 20 min at 2500 rpm (1,000 × g), 18◦ to 20◦C, with no brake;

Step 3.

Using a sterile pipet, remove the upper layer that contains the plasma and most\ of the remaining cell platelet fraction. Using another pipet, transfer the\ mononuclear cells layer to another centrifuge tube. This will appear as a white,\ cloudy band between the plasma and the Histopaque

Step 4.

Wash cells by adding 1X PBS (~3 times the volume of the mononuclear cell\ layer) and centrifuging 10 min at 450 to 600 × g, 18◦ to 20◦C.

Step 5.

Remove supernatant, resuspend cells in 1X PBS, and repeat the wash once to\ remove any remaining platelets

Step 6.

Resuspend mononuclear lymphocyte cells in complete RPMI-1640. Count cells and determine viability by trypan blue exclusion. If desired, purity of PBMC population can be determined by flow cytometry

Step 7.