INTRODUCTION
The Normal Peripheral Blood Mononuclear Cells (PBMCs) consist of the lymphocyte and monocyte fraction of whole blood. The cells are isolated from adult peripheral blood of healthy donors at IRB-approved and FDA-licensed blood banks. The mononuclear cells derived from adult peripheral blood contain large numbers of mature immune cells, thereby qualifying them for a diverse number of cell-based experiments.

The ImmunoPure HD Human PBMCs are isolated from fresh human adult peripheral blood of healthy donors at IRB-approved and FDA-licensed blood banks, using apheresis and immunomagnetic separation techniques.

MATERIALS AND METHODS
A. General Medium Requirement
   RPMI 1640 medium + 10% (v/v) FBS, 2 mM glutamine, 1% (v/v) nonessential amino acids, 1% (v/v) sodium pyruvate, 50 U/ml penicillin, 50 mg/ml streptomycin, and 50 mg/ml kanamycin.

B. Thawing Cells
   1. Prepare a 37°C water bath.
   2. Keep all samples frozen until the bath is ready.
   3. Place the vials into the water bath, being careful not to submerge below the junction between the lid and the vial.
   4. Place vial of cells in 37°C water bath and agitate until thawed. It is important to thaw the cells quickly; do NOT allow thawed cells to remain in freezing media any longer than necessary.
   5. When only a small amount of ice remains, remove the vials and dry with a lab tissue. Clean the top of the vial with a lab tissue moistened with 70% alcohol; avoid wiping away the labeling.
   6. Within about 30 sec., slowly add one milliliter of medium (containing serum) to the thawed cells.
   7. Slowly add thawed cells to 8 mL of medium containing serum. Invert tube 2 or 3 times to mix or mix gently by pipetting up and down several times.
   8. Centrifuge for 5 min at 400 x g.
   9. Aspirate or decant the supernatant and gently resuspend the cell pellet in 10 mL of medium.
   10. Remove an aliquot for cell count and proceed with experimental manipulations.
   11. Culture the cells in in RPMI 1640 medium supplemented with 10% (v/v) FBS, 2 mM glutamine, 1% (v/v) nonessential amino acids, 1% (v/v) sodium pyruvate, 50 U/ml penicillin, 50 mg/ml streptomycin, and 50 mg/ml kanamycin.

NOTE: The cell suspension may form clumps after standing at room temperature. This can be avoided by preparing and using the cells promptly or by adding DNase to the suspension at a final concentration of 10 units per ml.
The purchase price paid for the ImmunoPure™ HD cells and reagents grants end users a non-transferable, non-exclusive license to use the kit and/or its components for internal in vitro research use only as described in this manual; in particular, “research use only” excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis. Separate licenses are available for non-research use or applications. The PrimaPure™ HD cells and reagents are not to be used in human diagnostic or therapeutic applications, including primary or secondary use to produce or derive, directly or indirectly, any components used as drugs for human or animal use. Although routinely tested for HIV-1, HBV, HCV, Syphilis, and other infectious diseases, these cells must be handled as potentially infectious. There is no test that can completely guarantee the absence of infectious agents; care and attention should be exercised in handling the ImmunoPure cells by following standardized research lab practices, wearing protective lab clothing, and using appropriate equipment.

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