CHARACTERIZATION INFORMATION

Donor Demographics
- Age, Gender, Ethnicity, Height, Weight
- Date of Diagnosis
- Current Medications

INTENDED USE
Cryopreserved HIV Treatment Naive Human Peripheral Blood Mononuclear Cells (PBMCs) are available as controls and ex vivo model systems for cell based assays, flow cytometry assays and immune response monitoring.

For Research Use Only,
Not for use in diagnostic or therapeutic procedures.

PRODUCT DESCRIPTION
Cells are isolated from human donors with HIV who are treatment naïve using a density gradient centrifugation method for purification and minimizing red blood cell content. Cells display ≥70% viability post-thaw. The expected post-thaw cell recovery is ≥70% of the nominal cell count. Cells are provided in 1.5 mL of freezing media containing 10% DMSO.

INSTRUCTIONS FOR THAWING AND CULTURE
1. Transfer frozen vials to the lab on dry ice to keep them cold. Only thaw 4 vials at one time.
2. Warm media for cell dilution to 37 ± 3°C. For each vial to be thawed, place 10 mL of media in a 50 or 15 mL centrifuge tube.
3. Thaw the frozen vials in water in a 37 ± 3°C water bath with gentle shaking. Just before the last ice crystal has melted, remove the vial from the water. Wipe the vial with a sterile alcohol pad, focusing on the cap area.
4. Add 1 mL of warm culture media from the 50 or 15 mL tube prepared above to the vial using a 1000 µL pipette tip and drop-wise action. Add the culture media over 30 sec to allow the cells to adjust to the change of temperature.
5. Slowly transfer the diluted cells back to the 50 or 15 mL tube. Do not mix or pipette the cells vigorously since cells that have been frozen are initially more sensitive to mechanical stress than fresh cells.
6. Rinse the original vial with 1 mL of the cell containing media from the 50 or 15 mL tube to recover cells that may have adhered to the sides; add the rinse media back to the 50 or 15 mL tube.
7. Pellet the cells by centrifugation at 350g for 10 minutes with rapid acceleration and brake on. If no pellet is observed, centrifuge at 450g for an additional 15 minutes. Discard supernatant.
8. OPTIONAL STEP, PROCEED DIRECTLY TO STEP 9 IF PLANNING TO OMIT DNase TREATMENT: To avoid any issues with potential cell clumps, resuspend the cell pellet in a small volume of warm media (1-2mL) and mix by gently tapping. Add 100µL of 1mg/mL DNase I. Mix gently but thoroughly and incubate at room temperature for 15 minutes. Then bring the total volume to the desired counting volume using warm media, mixing the cell solution carefully. Note that if using these optional instructions, DNase does not need to be washed out of the cells, so proceed directly to Step#11 and perform your cell viability count.
9. Re-suspend the cell pellet by gently tapping and add 10 mL of warm culture media for a second wash. Mix cell solution carefully. Pellet the cells by centrifugation as in step 7.
10. Discard supernatant. Re-suspend the cell pellet by gently tapping and add 5-10 mL of warm culture media.
11. Count cells using laboratory specific procedures and proceed with laboratory protocol assays.

RESTING PERIOD FOR CELLS
Thawed cells may benefit from resting overnight at 37 ± 3°C before use in assays. Adjust viable cell concentration to 2 x 10^6 cell/mL. Put 1-10 mL of cell suspension in a 50 mL conical tube, loosen the tube cap to allow for gaseous exchange, and place the tubes upright in an incubator with the appropriate CO₂ level for the media used. After overnight incubation, count and use cells as needed.

REFERENCES
1. CDC recommendations for prevention of HIV transmission in health care settings. MMWR 36 (supp.2) 1987.