PBMC Thawing Protocol:

Reagent:

PBMC washing medium; A:RPMI-1640 with 5 to 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 1 mM sodium pyruvate, 2 mM L-glutamine;
B:50% X-vivo 15 medium (Lonza) + 25U/ benzonase,
C: 1X CTL-Anti-Aggregate-Wash™ (CTL))
PBMC complete culture medium: RPMI-1640 with 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 1 mM sodium pyruvate, 2 mM L-glutamine.

Protocol:

1. Warm Washing buffer and medium to 37°C in a water bath.
2. Remove vials from liquid nitrogen and transport them to the lab on dry ice.
3. Thaw frozen vials, only 1 vial at a time, in a 37°C water bath. When cells are nearly completely thawed, carry the vials to the hood and swab them with 70% EtOH.
4. Gently remove PBMCs (avoid pipetting up and down, as the cells are very fragile at this stage) and transfer the cells into a 50 mL falcon tube (Fisher scientific #14-432-22) containing 25 mL warmed washing buffer.
5. Use 1mL washing medium to rinse out the cryovial and gently mix the cells by inverting the 15 mL Falcon tube ~5x.
6. Wash 1: Spin the cells: 400x*g* for 8 min at room temperature. Pour off the supernatant.
7. Wash 2:

7.1 Suspend the cell pellet in 1 mL prewarmed medium (dropped slowly along the side of the tube) and resuspend the cell pellets, add 9 mL complete medium then Spin the cells 400*xg* for 8 min at room temperature.

7.2 If cells were thawed in the presence of benzonase, perform an additional wash with culture medium in the absence of benzonase.

8. Count cells and determine viability by Trypan blue staining