
Preparing Frozen PBMC for Use in the Evercode Assay

It's common for PBMCs sourced from a vendor to be already isolated, purified, and cryopreserved. Before being used in the Parse Biosciences Evercode™ v3 Fixation or Parse Biosciences Evercode™ Low Input Fixation workflow, the PBMCs must be prepared to maximize integrity and viability and to minimize shock that could potentially alter gene expression. Below is an internally validated protocol to prepare cryopreserved PBMCs for use in the Parse Biosciences Evercode™ assay platform.

1. Warm [RPMI-1640 media](#) with 10% FBS added to 37°C (50mL per sample). You will also need to aliquot some to keep at 4°C (10mL per sample).
2. Take a tube of PBMCs out of liquid nitrogen and thaw in a 37°C water bath only until no ice remains in the sample (~2 min).
3. Add thawed PBMCs to a 50 mL Falcon® tube.
4. Slow dilution of all samples in 37°C [RPMI-1640 media](#) with 10% FBS media.
 - a. Add 1 mL of warm media dropwise to the cells (1 drop for every 5 seconds)
 - b. Add 2 mL of warm media at a rate of 1 mL/5 seconds. Wait 1 minute
 - c. Add 4 mL of warm media at a rate of 1 mL/5 seconds. Wait 1 minute
 - d. Add 8 mL of warm media at a rate of 1 mL/5 seconds. Wait 1 minute
 - e. Add 16 mL of warm media at a rate of 1 mL/5 seconds. Wait 1 minute
 - f. Add 18 mL of warm media at a rate of 1 mL/5 seconds.

5. Spin down 200xg for 10 mins at 4°C. Remove all supernatant except for ~1 mL.
6. Resuspension in ~9 mL of 4°C [RPMI-1640 media](#) with 10% FBS.
7. Spin down 200 x g for 10 minutes at 4°C. Remove the entire supernatant, being careful not to disturb the pellet.
8. Resuspend cells in 100 µL Cell Prefixation Master Mix as prepared per the Evercode Cell Fixation User Guide.
9. Dilute 1:2 to count cells.
10. Finally, move up to the maximum number of cells* allowed by the Evercode Fixation kit into the cell fixation workflow.

*Up to 1 million cells per reaction for Evercode Cell Fixation v3 and up to 100,000 cells for Evercode Low Input Cell Fixation v3



Note: Please check the [Parse Support Suite](#) for the most up-to-date [User Guides](#).