Best Practices for Cell Preparation

Cell handling can directly impact phenotype making it critical to minimize cellular stress throughout the cell preparation workflow. This document contains tips and guidelines for optimized cell preparation prior to running your samples with the Pixelgen Proxiome kit. It serves as an extension of the UM001: User Manual Proxiome Kit Cell Surface (v1.01).

Platelet removal

To ensure high purity for samples isolated from blood, effective removal of platelet contamination is a critical step before fixation, as significant contamination can interfere with downstream results.

We recommend preventing this by performing multiple rounds of gentle washing to separate larger cells from smaller, lighter platelets. Centrifuge your samples at 120 x g for 10 minutes with the centrifuge brake turned off. This gentle force pellets your target cells while leaving the majority of platelets in the liquid supernatant. Repeat this slow centrifugation wash 2-3 times for optimal purity.

Red blood cell (RBC) removal

If the samples have significant RBC contamination we recommend removing them before proceeding with the fixation step as they can interfere with the final results. Efficient RBC lysis can be achieved using e.g. the RBC lysis buffer 10x (eBiosciences, Cat. no. 00-4300-54).

Cell clumping

For the most accurate cell counts, it is important to remove any visible cell aggregates or debris before fixation. We recommend visually inspecting your sample and, if clumps are present, filtering the cell suspension through a cell strainer (e.g., 40-70 µm) to remove them.

Cell dissociation

Some in-vitro cultured immune cells may adhere to plastic and might require dissociation before fixation. We have successfully dissociated activated T-cells and breast cancer tumor-spheroids using Accutase for 15 min @ 37°C with pipetting every 5 min. While some epitopes might be lost, we found Accutase to be gentler than trypsin.

Dead cell removal kit

If during cell preparation viability is lower than 70% we recommend using a dead cell removal kit. High amounts of dead cells in the samples can impact the downstream results. We recommend using: EasySep Dead Cell Removal (Annexing V) kit from Stem Cell Technologies (Cat. no. 17899) or Dead Cell Removal Kit From Milteny Biotec (Cat. no. 130-090-101).

Rare cell types

The maximum number of cells/sample to input to the sequencing step with the Proximity Network assay is 1000 cells. If your cells of interest are rare (<5%), an enrichment step before fixation may be required. This can be achieved using positive/negative selection with magnetics beads or FACS sorting. When choosing the strategy, please be aware that positive selection might use an antibody that can block the target epitope needed for detection with the Proxiome kit.