# INSTRUCTIONS FOR USE

- Please plan your time frame for cell preparation according to the given submission schedule with the Single-cell Center.

- Minimize delays between cell preparation and submission. Keep the cells on ice.

- Solid tissues and other large cell aggregates must be dissociated with mechanical or enzymatic dissociation.

- Be very careful with your cell preparation (pipette slowly).

- To minimize shear forces during pipetting, use 10X Genomics validated large diameter 1000 µl pipette tips for cell manipulation and resuspension (Rainin cat#30389218).

- It is recommended to perform an initial cell count before transferring the cells to the Single-cell Center to determine cell concentration and cell viability.

- Cell filtration can be performed to ensure that a well single-cell suspension is free of cell debris and cell aggregates. Ensure that the pore size is larger than the diameter of the cells but small enough to trap clumps and debris. Take into account the volume loss associated with the filtration process and repeat the cell count to correct for cell loss.

- Submit cell samples with a concentration of <2000 cells/µl (2 million cells/ml). Excessive cell concentration may lead to aggregation and clumping, which may interfere with the generation of ideal single-cell suspensions. Target value for cell concentrations is between 1000 and 2000 cells/µl (1 million to 2 million cells/ml).

- Target value for the cell suspensions is a viability of >90%. Lower cell viability reduces the apparent efficiency of cell division and recovery, as non-viable and dying cells generally contain less RNA, which is more fragmented.

- Cell viabilities of <70% will not be processed for single cell library preparation.

- Do not over-centrifuge cells during pelleting. Recommended centrifugation conditions are 150 x g for 3 minutes at room temperature for larger cells (e.g. immortalised cell lines) and 300 x g for 5 minutes at room temperature for smaller cells (e.g. PBMCs).

- The recommended solution for cell washing and resuspension is 1xPBS (calcium and magnesium free) with 0.04% w/v BSA (400 µg/ml). For other sensitive cell types, washing and resuspension can be done in alternative buffers (10X validated - DPBS & HBSS).