Processing Leukapheresis Samples for Specific Cell Isolation Without Density Centrifugation

Preparing PBMC from leukapheresis samples by density centrifugation for later cell isolation is time-consuming and requires precision. EasySep™ negative selection kits from STEMCELL Technologies allow you to isolate untouched cells directly from leukapheresis samples, without the need for density centrifugation. This protocol demonstrates how to prepare the leukapheresis samples for downstream cell isolation.

Protocol

If working with large volumes (> 150 mL), first concentrate the leukapheresis cells by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukopak volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium).

For small volumes (< 150 mL), add the Ammonium Chloride Solution directly to the cell suspension.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis suspension (e.g. for 5 mL of leukapheresis suspension, add 5 mL Ammonium Chloride Solution).
2. Incubate 15 minutes on ice.
3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25 ° C). (G to RPM Converter)
4. Remove the supernatant.
5. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x g for 10 minutes at room temperature (15 - 25 ° C) with the brake off. Carefully remove the supernatant.
6. Repeat the wash step one or more times until most of the platelets have been removed (indicated by a clear supernatant).
7. Resuspend cells at the recommended cell concentration, in recommended medium.