Creighton
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Preparation of White Cell Suspension by Lysis of Erythrocytes

(McCoy, J.P., 1998. Handling, storage and preparation of human blood cells. *Curr. Protocol. Cytom.* 5:5.1.1-5.1.13)

Although erythrocytes can be separated from mononuclear cells by density-gradient separation, many laboratories prefer lysis methods to eliminate erythrocytes from various specimens. Lysis is much quicker than gradient separation and in general leaves the remaining white cell populations relatively unperturbed. Blood samples may be treated with any anticoagulant.

This procedure, in which erythrocytes are lysed with ammonium chloride, may be used for unstained blood or blood that has already been incubated with monoclonal antibodies. In general, this method will not affect the pattern of staining observed for most lymphoid markers. The viability of white blood cells subjected to this treatment is good.

Commercial reagents such as FACSLyse, ImmunoLyse, and Optilyse can be used in place of ammonium chloride to lyse erythrocytes. However, as FACSLyse contains a fixative, staining for cell surface markers on leukocytes should be performed prior to lysis of the erythrocytes with this reagent. Stain whole blood with antibodies, then lyse erythrocytes according to manufacturer's instructions. Optilyse may be used without subsequent washing of the leukocytes.

Equipment:

Centrifuge
12x75mm tube(s)

Reagents:

Phosphate Buffered Saline (PBS)
1x Ammonium Chloride Lysing Solution (prepared fresh from 10x stock), or
FACSLyse, ImmunoLyse or Optilyse

Method:

1) Place 200 µl whole blood sample in a centrifuge tube and add 3 ml fresh 1x ammonium chloride lysing solution. Incubate 10 min at room temperature.
2) Centrifuge 5 min at 300 x g, room temperature (22º to 25º C).
3) Discard supernatant. Resuspend cells in 2 ml PBS. Centrifuge 5 min at 300 x g, room temperature.
4) Discard supernatant and repeat wash with 2 ml PBS.
5) Resuspend cells as necessary for specified assay. Use immediately or store as indicated for assay.