

A Method for Lysing Red Blood Cells In Vi-CELL Sample Preparations

Blood cell viability measurements on the Beckman Coulter Vi-CELL will almost entirely involve analyses of the White Blood Cell population. Mature Red Blood Cells are “stripped down” cells without nuclei designed to efficiently deliver oxygen. The cells analyzed will be lymphocytes, monocytes, neutrophils, basophils, or eosinophils. Hence, the Red Blood Cell population must be “removed” through use of an appropriate lysing agent.

Most common lytic agents, Zap-Oglobin, for example, quite effectively lyse red cells; however, they also remove the cell membranes of the remaining White Blood Cells. Thus, the entire WBC population will erroneously appear NON-VIABLE due to uptake of the trypan blue stain. Therefore, when lysing RBC's in whole blood or bone marrow samples, for instance, a lytic agent must be selected which has no detrimental effects on the cellular membranes of the White Blood Cells.

Ammonium chloride (NH_4Cl) is one choice for an effective RBC lysing agent for Vi-CELL samples. The product may be purchased from StemCell Technologies(1-800-667-0322), Inc. in Vancouver, BC, Canada.

The procedure for cell preparation using ammonium chloride lyse is as follows:

1. Add 100 microliters of cell suspension to a 12 X 75 mm tube.
2. Add 2 mls. Of the ammonium chloride lyse, vortexing immediately.
3. Let sit at room temperature (in the dark) for 6-10 minutes.
4. Vortex. Centrifuge at 1400 rpm for 5 min.
5. Aspirate the supernate and vortex the pellet.
6. Add 2 mls of Isoton II. Vortex.
7. Centrifuge at 1400 rpm for 5 min.
8. Decant, and vortex the pellet.
9. Add tissue culture media or Isoton II for analyses on the Vi-CELL