SOP-P041

Chicken RBC - Sterile Preparation for Flow Cytometric DNA Determination

Objective: To prepare chicken red blood cells for use as internal standard for DNA analysis.

NOTE: **(a) Perform the following procedures aseptically in the sterile hood.**
(b) Call **Donna Schrader** at ADDL ph**# 4-7454** for availability of chicks and to make an appointment.

Procedure:

Cell Collection:

1. Prepare a 5ml syringe with a 22 gauge needle.
2. Draw a very small amount of heparin (approx. 0.25ml) into the syringe.
3. Draw approx. 0.25ml of sterile saline into the syringe (amount not critical).
4. Wet the inside of the syringe well.
5. Exchange the 22 gauge needle with an 18 gauge x 1 ½" needle.
6. Take the prepared syringe and 70% ethanol in a squirt bottle to ADDL.
7. Donna will remove some of the feathers and the wet the area with the 70% ethanol prior to cardiac puncture. Any amount of cRBC's up to 5ml will be sufficient.
8. Invert the syringe gently approximately 10x to mix the cells and anticoagulant.
9. Return to the lab.

Cell Preparation:
1. Divide the volume of blood equally into two 15cc conical tubes.
2. Centrifuge cells at 400xg for 10 minutes at 4°C.
3. Remove plasma using a sterile pasteur pipet, and carefully aspirate buffy coat. Discard.
4. Resuspend with approximately 10 ml cold sterile saline, mix gently to make sure the button is dispersed.
5. Centrifuge as above and discard wash solution.
6. Repeat steps 3 and 4 twice (3 times total).
7. Resuspend cRBC's in approximately 5 ml sterile saline solution, keep at 4°C.

Cell Enumeration:

A. Run a hematocrit on the cell prep and concentrate or dilute to a cell concentration of 5%. This translates to a concentration of approximatley $4x10^8$ cells per ml.

Or

B. Do a coulter count:

(a) Prepare a 1:500 dilution of the cRBC's by pipetting 20µl of cell suspension into 10ml of PBS in a coulter counting vial. Mix gently by inversion.
(b) Pipet 100µl of the 1:500 dilution to 10ml of PBS in a new coulter vial. This is now a 1:50,000 dilution. Mix gently by inversion.
© Count on Coulter Counter (see SOP-P005)
(d) Adjust the cell concentration to 4×10^8 cells per ml.

Storage:

1. Aliquot the adjusted cRBC's into 1ml cryovials with screw cap lids.
2. Label with the date and cell concentration.
3. Store at 4°C.

Working dilutions:

1. Use 10^5 cRBC's for each 10^6 test sample.
2. For a concentration of 0.02% or approximately 1.5x 10^6 cells per ml:
   Dilute stock cRBC's 1:250 (20µl cRBC + 4.98ml diluent)
3. Use 100µl of this working cRBC dilution for each 1ml of 10^6 test sample.

Alternate dilution:

For a concentration of 1.0x10^6/ml:
16.5µl cRBC's to 4.4ml PBS.

Discussion:

When kept sterile, fresh cRBC's remained good (HPCV's less than 5.0) for approximately 3 months. When HPCV's are > 5.0, or when you see debris in the lower channels, fresh cRBC's must be prepared.

Created by: Kathy Ragheb **Date:** April 30, 1999

Verified by:_____Date:_____ _____

Print Name Sign Name