CALTAG LABORATORIES

FETAL HEMOGLOBIN DETECTION, TIPS & TRICKS

FEATURES

FETAL HEMOGLOBIN KIT

- FDA cleared
- Fluorescent antibodies to hemoglobin F
- FITC, PE & TRI-COLOR®
- Sensitivity < 0.1%
- Linearity > 98%
- Excellent correlation with slide (Kleihauer-Betke) test
- Easy fixation and permeabilization protocol

FETALTROL™ CONTROL CELLS

- FDA cleared
- Values close to expected clinical range
- Great for establishing assay and setting markers

FIXATION

- Stabilizes red blood cell (RBC) membranes and cross-links surface antigens.
- Glutaraldehyde must be fresh and stored properly at -20° C. Caltag suggests the use of 1.0 ml ampules from Sigma (Cat. No. G5882), shipped and stored frozen (-20°C). Thaw individual ampules rapidly just before use (in hands, not water bath). Unused portion should be discarded.
- Prepare a 0.05% working solution using 1 x phosphate buffered saline (PBS), no azide or bovine serum albumin (BSA), as a diluent, keep cold.
- Adhere to recommended incubation time (minimum 10 minutes, no more than 12).
- Wash thoroughly. Do not skip any of the 3 washes.
- Poor separation is most commonly due to inadequate fixation or permeabilization.
- Some RBC lysis may be noted.

PERMEABILIZATION

- "Pokes holes" in RBC membranes. Allows antibody to enter cytoplasm.
- It is much easier to use Caltag's Triton X-100 from the kit when establishing procedure. Stock Triton X-100 is highly viscous and difficult to measure in small volumes.
- Dilute Triton X-100 to 0.1% (1:10 dilution) with PBS-0.1% BSA.
- Keep and use cold.
- Do not exceed 5 minutes incubation at room temperature.
- Wash once.

REMEMBER, FIX & PERM® CANNOT BE SUBSTITUTED IN THE PROTOCOL ABOVE

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L20045 REV. 02-02 DCO# 000659

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DATA ACQUISITION

- Set instrument to usual detector and compensation settings.
- Change FSC and SSC to log scale.
- Threshold on FSC signal.
- Compensation is not an issue if only using one color.
- Start with negative control. Adjust PMT voltage if needed (fixation and permeabilization protocol may increase autofluorescence). Keep negative peak in first decade.
- Collect 50,000 events, ungated unless there is significant debris.
- Run a rinse tube between samples to prevent carryover.
- Consider running negative (level one) control first, followed by patient samples, then positive controls.
- Remember that this is rare-event analysis and positivity is not easy to see during acquisition.

DATA ANALYSIS

- When using a FITC-conjugated antibody, set an analysis gate on FSC vs. FL2, excluding FL2 bright positives. These are WBC's with increased autofluorescence.
- Several continuous populations of cells may be seen in the FSC parameter.
- Analyze on a single fluorescence histogram. Start with high positive control first to get a clear picture to set markers.
- There should be a large negative peak with a "shoulder" consisting of F cell. These cells are normal adult RBCs experessing varying amounts of hemoglobin F. Set marker past shoulder, or around bright positives.
- True fetal cells are bright positive and will generate a separate peak, usually well separated in the third decade.
- Use known control values to determine marker positions.