CALTAG
LABORATORIES

Immunofluorescent Staining of Human Blood with Red Blood Cell Lysis

Solutions:

***Wash Medium*:** 2 % fetal bovine serum (FBS) + 0.1 % sodium azide in phosphate buffered saline (PBS)

***Red blood cell (RBC) lysing solution*:** 0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2-7.4 or Caltag's Cal-Lyse ™ whold blood lysing solution (Code # GAS010)

***Fixation solution*:** 1 % Paraformaldehyde in PBS

Procedure:

1. Add the appropriate amount of isotype control and specific antibody into labeled 12 x 75 mm tubes.
2. Pipette 100 µl (1 x 10^6 cells) of well-mixed anti-coagulated whole blood to each tube. Mix well and incubate for 20-30 minutes at 4°C in the dark.
3. Add 3 ml of RBC lysing solution per tube and mix by vortexing. Incubate at room temperature (RT) for 10 minutes.
4. Centrifuge for 3-5 minutes at 300 x g. Discard the supernatant. Resuspend the pellet by vortexing and wash two times with 3 ml of wash medium. For single step experiments proceed to step 7.
5. For indirect staining, add the appropriate amount of second step antibody to each tube. Mix well, and incubate for 20-30 minutes at 4°C in the dark.
6. Wash two times with 3 ml of wash medium.
7. Resuspend the cell pellet in 500 µl of fixation solution, or 500 µl of wash medium if samples are to be analyzed on the same day.
8. Perform flow cytometric analysis.

NOTES:

1. The Cy dyes and Texas Red ® (TR) are sensitive to aldehydes in fixation medium. It is important that samples stained with the tandem flurophores (fluors), TRI-COLOR® (TC, PE-Cy5), PE-Cy7, APC-Cy7 and PE-TR be analyzed within 18 hours of fixation. Higher than normal compensation values are indicative of degradation.
2. To block Fc receptor binding of antibody to human cells, and to reduce non-specific binding in general, pre-incubate the cells with normal serum or purified IgG from the same species as the specific antibody. For example, when using a mouse anti-human monoclonal antibody, block with mouse serum or purified mouse IgG. This can be done by incubating the cells for 10 minutes using 10 µg of purified IgG per one million cells. After 10 minutes, add the antibodies being used for immuno-fluorescent staining directly to the mixture of cells and blocking IgG. Incubate for the appropriate amount of time (see Appendix K for additional information).

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L20046 REV. 02-02 DCO# 000000