

CAL-LYSE™ STAINING PROTOCOL FOR FLOW CYTOMETRY

Description

Cal-Lyse is a premixed lysing solution specifically formulated to lyse erythrocytes following monoclonal antibody staining of whole blood or bone marrow. Treatment with this reagent leads to both the lysis of red blood cells and the fixation of white cells. Treatment does not affect fluorochrome-labeled antibodies bound to leukocytes and leaves morphological scatter characteristics of leukocytes intact. Cal-Lyse can be used for either “no-wash” or “wash” staining procedures. For certain antibodies, a wash step is strongly recommended.

Storage and Stability

Cal-Lyse should be stored at room temperature. The expiration date is stated on the package label. Do not use this reagent if a precipitate should form or if discoloration occurs.

Warning

Cal-Lyse solution contains formaldehyde (< 5%). Formaldehyde is toxic, allergenic and a suspected carcinogen. Avoid ingestion, inhalation, and contact with eyes, skin and clothing.

“No-Wash” Staining Procedure

1. Add antibody conjugate per the instructions of the antibody manufacturer.
2. Add 100 µl of whole blood and mix.
3. Incubate for 15 minutes at room temperature in the dark.
4. Add 100 µl of Cal-Lyse (kept at room temperature) and vortex.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 1 ml of deionized water (kept at room temperature), incubate for 5 minutes and vortex.
7. After an additional 5 minutes at room temperature, analyze cells on a flow cytometer or store the samples in the refrigerator until analysis is performed.

“Wash” Staining Procedure

1. Add antibody conjugate per the instructions of the antibody manufacturer.
2. Add 100 µl of whole blood and mix.
3. Incubate for 15 minutes at room temperature in the dark.
4. Add 100 µl of Cal-Lyse (kept at room temperature) and vortex.
5. Incubate for 10 minutes at room temperature in the dark.
6. Fill the tube and suspend cells with deionized water (kept at room temperature).
7. Spin the cells for 10 minutes at room temperature and remove supernatant.
8. Resuspend the cells in Sheath Fluid (e.g. 0.2 - 0.5 ml) and analyze or store in the refrigerator.

Note:

For the determination of surface Ig (SIG) it is recommended that the blood be washed before mixing it with the antibody conjugate. This recommended wash step is in addition to the wash step after lysis.

Flow Cytometry

Cal-Lyse is designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to the manufacturer’s instructions.

Typical Scatter Patterns

The following scatter patterns of a Cal-Lyse-treated whole blood sample were obtained using a BD FACSCalibur™ flow cytometer. The following profiles were generated with either a wash staining procedure or a no wash staining procedure as indicated.

