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NBP3-45118 Protocol

Product Handling Protocol (NBP3-45118)

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Background
Flow cytometric analyses with monoclonal antibodies were so far restricted to leukocyte populations, which had been separated from erythrocytes before staining and/or analysis. Instead, whole blood staining methods allow for a rapid and accurate determination of cellular subpopulations in non-separated biological samples. This is not only time saving but reduces also the probability of an unintended loss of distinct cellular populations due to e.g. commonly used differential centrifugation procedures. With the Flow Cytometry Lysis Solution, flow cytometric analysis of whole blood has become as easy and accurate as the analysis of separated cell populations. Results must be interpreted by a certified professional before final interpretation. Analyses performed with this antibody should be paralleled by positive and negative controls. If unexpected results are obtained which cannot be attributed to differences in laboratory procedures, please contact us.

Product
Flow Cytometry Lysis Solution can be applied in wash- or no-wash lysing procedures with whole blood or bone marrow samples.

Flow Cytometry Lysis Solution is a premixed, ready to use lysing solution fomulated for lysing erythrocytes following monoclonal antibody staining of whole blood. Treatment with this reagent simultaneously leads to lysis of red blood cells and fixation of white cells. Morphological scatter characteristics of leukocytes remain intact. Flow Cytometry Lysis Solution can be used with or without sample washing. Flow Cytometry Lysis Solution is suitable for the analysis of normal and malignant leukocyte populations derived from various human biological samples (blood, bone marrow and others) using flow cytometry. Results must be put within the context of other diagnostic tests as well as the clinical history of the patient by a certified professional before final interpretation.

The quality of each lot is determined by lysing red blood cells of well defined blood samples from representative donors and subsequent comparison of forward and side scatter characteristics of obtained leukocytes.

Applications
Biological fluids (blood, bone marrow, and others) must be collected under sterile conditions. Anticoagulation with EDTA or heparin is recommended. The samples should be stored at room temperature until used. For optimal results, samples should be processed and analyzed within 24 hours. Samples with high numbers of non-viable cells might cause false results, such cases require determination of cell viability with e.g. propidium iodide. All biological samples have to be handled with caution. Always consider them as potentially infective. Use appropriate precautions such as gloves, lab-coat, etc.

No-wash staining and lysing procedure
1. For each sample add 50 ul of EDTA anti-coagulated blood to a 3-5 ml tube
2. Add 20 ul of the appropriate monoclonal antibody conjugate
3. Incubate the tube for 15 minutes at 4 degrees C or at room temperature in the dark
4. Add 100 ul Flow Cytometry Lysis Solution to each tube and incubate for 10 minutes at room temperature
5. Add 1 ml of distilled water and vortex, incubate for 5-10 minutes at room temperature
6. Analyze immediately or store samples at 2-8 degrees C in the dark and analyze within 24 hours

Wash staining and lysing procedure
1. For each sample add 50 ul of EDTA anti-coagulated blood to a 3-5 ml tube
2. Add 20 ul of the appropriate monoclonal antibody conjugate
3. Incubate the tube for 15 minutes at 4 degrees C or at room temperature in the dark
4. Add 100 ul Flow Cytometry Lysis Solution to each tube and incubate for 10 minutes at room temperature
5. Add 3-4 ml of destilled water and vortex, incubate for 5-10 minutes at room temperature
6. Centrifuge tube for 5 minutes at 300 g
7. Aspirate supernatant and resuspend pellet in 0.3 ml of sheath fluid
8. Analyze immediately or store samples at 2-8 degrees C in the dark and analyze within 24 hours

Flow Cytometry Lysis Solution is designed for use with all commercially available flow cytometers. Alignment and

compensation should be performed according to manufacturer'ss instructions.

Limitations of the technique: Flow cytometry should be performed by professional users only. Improper alignment of the flow cytometer, inaccurate compensation of fluorescence leaking into other channels as well as incorrect positioning of regions may lead to false results. Lysis of red cells might be impossible for various reasons. In such instances it is recommended to isolate mononuclear cells (MNC) via density gradient centrifugation prior to staining. Results will be correct and reproducible as long as the procedures used respect the technical recommendations and obey good laboratory practice. The Flow Cytometry Lysis Solution is provided at a concentration that will allow lyse human erythrocytes. It is therefore strongly recommended to stick to the working protocol in terms of concentration and volume regarding cells and antibody. The properties of Flow Cytometry Lysis Solution have been determined using EDTA anti-coagulated peripheral blood. Avoid ingestion and inhalation and contact with eyes, skin and clothing. Proper handling procedures are recommended.

Storage
Flow Cytometry Lysis Solution should be stored and used at room temperature. Stability of the reagent: Please refer to the expiry date printed onto the vial. The use of the reagent after the expiration date is not recommended. Do not use reagent if a precipitate should form or discoloration occurs. If unexpected results are obtained which cannot be attributed to differences in laboratory procedures, please contact us.