PerFix-nc Kit (no centrifuge assay Kit)

For Intra- & Extra-Cellular Staining Preparation

PN B10825 – 75 tests – Liquid

For Research Use Only. Not for use in diagnostic procedures.

REAGENTS KIT COMPONENTS

- PerFix-nc Buffer 1, Fixative Reagent: 1 vial (1.9 mL, Liquid) containing Formaldehyde – 5 or 25 µL/test
- PerFix-nc Buffer 2, Permeabilizing Reagent: 1 vial (22.5 mL, Liquid) containing Proclin 300 – 300 µL/test
- PerFix-nc Buffer 3, Final 10X Solution: 1 vial (26.3 mL, Liquid, 10X Concentrated) containing Formaldehyde – 300 or 350 µL/test

DESCRIPTION

The PerFix-nc Kit (no centrifuge assay Kit), for Intra- & Extra-Cellular Staining Preparation, consists of two ready-to-use reagents, and one reagent requiring a 10-fold dilution before use. Its purpose is to induce permeability in the cytoplasmic and nuclear membranes of leucocytes for the demonstration of intracellular antigenic determinants by means of dyes or fluorochrome-conjugated antibodies. PerFix-nc Kit can be used to prepare biological samples for analysis by flow cytometry. It has been developed to enhance the signal-to-noise ratio of cellular staining and to simplify the workload necessary for the sample preparation. Accurate detection of both intracellular and extracellular epitopes are obtained, while:

- There are no washing steps through the procedure. Only a final wash step is optional.
- Several surface markers can be added together with the intracellular markers and incubated simultaneously to the permeabilization step.
- Total duration of the procedure and total workload are similar to current procedures for surface staining.
- Automation of the procedure is rendered possible thanks to the removal of the washing steps.

REAGENT

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REAGENT CONTENT

- PerFix-nc Buffer 1 – Fixative Reagent PN B10827 - 75 tests - Liquid
- PerFix-nc Buffer 2 – Permeabilizing Reagent PN B10828 - 75 tests - Liquid
- PerFix-nc Buffer 3 – Final 10X Solution PN B10829 - 75 tests - Liquid

APPLICATION

Flow cytometric analysis of multiple intracellular epitopes, or both multiple intracellular and cell surface markers, by fixation, erythrocyte lysis and permeabilization of human leucocytes from peripheral whole blood.

PerFix-nc Kit has been optimized for the preparation of human whole blood, but could be used also with mouse blood and total human bone marrow.

STATEMENTS OF WARNING

1. PerFix-nc Buffer 1 (Fixative Reagent) and Buffer 3 (Final 10X Solution) contain formaldehyde. Formaldehyde is toxic and allergic and is considered as a carcinogenic agent. Handle with care in well ventilated areas. Never pipet by mouth, avoid all contacts with skin, mucous membranes, eyes and clothing (wear protective gloves, glasses and gown).
2. PerFix-nc Buffer 2 (Permeabilizing Reagent) contains Proclin 300. Proclin-300 is a potentially irritating compound. Handle with care in well ventilated areas. Never pipet by mouth, avoid all contacts with skin, mucous membranes, eyes and clothing (wear protective gloves, glasses and gown).
3. Biologic hazard: all blood samples may potentially harbor infectious agents. Universal precautions must be taken in all steps of this procedure involving blood or its derivatives. In particular, specimens, samples, and all material coming in contact with them should be considered potentially infectious. These materials should be disposed off with proper precautions.
4. Never pipet by mouth and avoid contacts of samples with skin and mucous membranes.
5. Do not use reagents beyond the expiration date shown on the label.
6. Do not expose reagents to strong light during incubation.
7. Avoid microbial contamination in the reagents or incorrect results might occur.
8. Use good laboratory practices when handling the PerFix-nc buffers and all other reagents.

STORAGE CONDITIONS AND STABILITY

PerFix-nc Buffers are stable up to the expiration date shown on the label when stored at room temperature (18 – 25°C). Do not freeze. If the PerFix-nc Buffer 3 (Final 10X solution) is stored below 10°C, crystals may appear; if this is the case, allow it to return to room temperature and verify the complete redissolution.

METHODOLOGY

KIT REAGENT PREPARATION

1. No reconstitution is necessary. Both reagents may be used directly from the vial.

PerFix-nc Buffer 3 Reagent preparation

Dilute the 10X Concentrated PerFix-nc Buffer 3 (Final 10X Solution) into deionized water: 1 volume of Buffer 3 with 9 volumes of water. Mix well before use. We recommend preparing only the volume of Final 1X Reagent necessary for the experiments of the day. Stability of the Final 1X Reagent, as diluted from Buffer 3 has not been thoroughly evaluated.

MATERIAL REQUIRED BUT NOT PROVIDED

Specimen for testing: Whole blood in anti-coagulant tube. The blood specimen must be used as soon as possible in order to preserve epitopes and cell structures (within 24h), but depending on the application, some older specimens can be analysed.

Equipment:

- Pipetors to deliver from 1 to 1000 µL
- 5 mL plastic tubes
- Timer
- Automatic agitator (Vortex type)
- Fluorochrome-conjugated antibodies against intracellular epitopes and surface molecules
- Centrifuge, variable speed (if using washing step)
- Flow Cytometer

PROCEDURE

1. Pipet 50 µL of blood sample into the bottom of each appropriately labeled tube. Avoid putting some blood on the side of the tube; otherwise it will not be appropriately treated.
2. Pipet 5 µL of the Fixative Reagent to each tube (alternatively use 25 µL, see Special Note No.3).
3. Vortex immediately and incubate for 15 min at room temperature (18 – 25°C).
4. Vortex again the fixed blood and add 300 µL of the Permeabilizing Reagent to each tube; vortex immediately.
5. Add immediately to each tube the fluorochrome-conjugated antibodies against intracellular epitopes and surface molecules (alternatively, the antibodies can be pre-mixed into the Permeabilizing Reagent and added altogether at the end of the fixation step).
6. Vortex immediately and incubate for 15 – 30 min at room temperature.
7. Add 3 mL of the Final 1X Reagent (prepared from the 10X concentrated Final Solution) to each tube; vortex immediately; the sample is now ready for analysis on a flow cytometer.

SPECIAL NOTES
1. All conjugated antibodies, for intra- and extra-cellular staining, must be titrated for optimal results.
2. For some surface markers, even the relatively low fixative concentration implemented in the PerFix-nc Fixative Reagent could be detrimental to the surface epitopes. In this case, it is recommended to either choose another antibody clone or another marker (if possible) or to add the affected antibodies to the specimen before the fixation step, and pre-incubate with blood the recommended time.
3. On the contrary, for some intracellular epitopes, the relatively low fixative concentration is not sufficient to retain the molecule inside the cell (especially for small soluble antigens). In this case it is recommended to apply a higher fixation by using 25 µL of the Fixative Reagent instead of 5 µL. The rest of the procedure remains the same. Surface markers reactivity should be re-evaluated in these conditions. NOTE: the kit contains enough Fixative Reagent to perform this higher fixation procedure for all 75 tests. For any new intracellular marker, it is recommended to titrate the new antibody in parallel with the two procedures (normal and high fixation), and choose the optimal condition.
4. Optional final wash step: an optional centrifugation step is possible at the end of the procedure; it will allow concentrating the cells for a faster analysis on the flow cytometer and eventually will improve the signal-to-noise ratio (typically by 20% to 50%): pellet the cells at 500 x g for 5 minutes, and resuspend them in the same Final 1X Reagent (recommended 0.5 mL).
5. Some markers continue to react in the Final 1X Reagent (e.g. anti-MPO), therefore an accurate comparison between multiple specimens would require to match all the incubation times, including in the Final 1X Reagent. Ultimately, a timed start could be required.
6. Cells in the Final 1X Reagent are stabilized by formaldehyde. Despite some common small variations in the staining and cell structure, it is possible to analyze the samples after 24h. It is then recommended to store the tubes at 2 – 8°C.
7. For some rare blood samples, the RBC lysis may not be complete immediately after the addition of the final buffer. If debris of RBC are significantly contaminating the SS/FS histogram, it is recommended to allow the sample for a longer incubation (typically 10-30 min more) and restart the analysis on the flow cytometer.
8. When possible, it is useful (and recommended) to batch the tests:
   a. Multiple experiments on the same blood specimen will benefit from a batch fixation (upscale blood volume and fixation buffer as necessary, then distribute e.g. 55 µL (50 µL blood + 5 µL Fixative Reagent) or 75 µL (50 µL blood + 25 µL Fixative Reagent if using a higher fixation) into the test tubes during the 15 min incubation time (or at the 15 min time point if the tubes already contain the Permeabilizing Buffer + antibodies mix).
   b. Multiple experiments on different specimens but with the same antibody cocktails will benefit from a batch preparation of the Permeabilizing Buffer + antibodies. In this case distribute 300 µL + antibodies volume into the test tubes after the 15 min fixation time (or during the batch fixation).
9. PerCP dyes may not perform optimally with PerFix-nc Kit.