PerFix EXPOSE (Phospho-Epitopes Exposure kit)

PN B26976 – 75 tests – Liquid

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

REAGENTS KIT COMPONENTS

PerFix EXPOSE Buffer 1, **Fixative Reagent:** 1 vial (3.75 mL / vial, Liquid) - PN B26977
PerFix EXPOSE Buffer 2, **Permeabilizing Reagent:** 3 vials (25 mL / vial, Liquid) - PN B26978
PerFix EXPOSE Buffer 3, **Staining Reagent:** 2 vials (3.75 mL / vial, Liquid) - PN B26979
PerFix EXPOSE Buffer 4, **Final 20X Solution:** 1 vial (26.25 mL / vial, Liquid, 20X Concentrated) - PN B26980

DESCRIPTION

The PerFix EXPOSE Kit (Phospho-Epitopes Exposure kit) is a Fast & Easy Procedure for Cell Signaling. It consists of three ready-to-use reagents, and one solution requiring a 20-fold dilution before use. Its purpose is to induce permeability in the cytoplasmic and nuclear membranes of leucocytes or human cell lines for the detection of phosphorylated intracellular antigenic determinants by means of dyes or fluorochrome-conjugated antibodies.

PerFix EXPOSE 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 most intra-cellular phospho-epitopes stainings and to simplify the workload necessary for the sample preparation. Accurate detection of both intracellular and extracellular epitopes is obtained, while:

- There are only two washing steps through the procedure.
- There is no methanol treatment, and therefore no ice incubation required.
- A unique procedure can be applied to all phospho-epitopes.
- Several surface markers can be added together with the intracellular markers and incubated simultaneously.
- Total duration of the procedure and total workload are similar to current procedures for surface staining (about 1 hour).

REAGENT

PerFix EXPOSE Kit (Phospho-Epitopes Exposure kit)
PN B26976 - 75 tests - Liquid

APPLICATION

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

A specific procedure is also described for samples containing no or few erythrocytes like Peripheral Blood Mononuclear Cells (PBMC) or cell lines.

PerFix EXPOSE Kit has been optimized for the preparation of human samples.

STATEMENTS OF WARNING

1. Buffer 1 contains formaldehyde.
2. Buffer 2 contains Proclin 300, sodium perchlorate, and detergent.
3. Buffer 3 contains Proclin 300, sodium perchlorate, and sodium azide.
4. Buffer 4 contains formaldehyde, and detergent.
5. All these compounds are either potentially irritating or harmful. Formaldehyde is toxic and allergenic and is considered as a carcinogenic agent. Sodium azide may form hazardous products when in contact with inorganic acids.
6. 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.
7. Use good laboratory practices when handling the PerFix EXPOSE buffers and all other reagents. 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).
8. Do not use reagents beyond the expiration date shown on the label.
9. Avoid microbial contamination in the reagents or incorrect results might occur.
10. Reagents and waste should be eliminated according to local requirements.

STORAGE CONDITIONS AND STABILITY

PerFix EXPOSE 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 EXPOSE Buffer 2 or Buffer 4 is stored below 10°C, cristals may appear; if this is the case, allow it to return to room temperature and verify the complete redissolution. Gently mix when redissolution is complete.

METHODOLOGY

KIT REAGENT PREPARATION

PerFix EXPOSE Buffer 1, 2 and 3 are ready to use. The PerFix EXPOSE Buffer 4 (Final 20X Solution) need to be diluted 20X into deionized water: 1 volume of Buffer 4 with 19 volumes of water**. Mix well before use**. We recommend preparing the volume of Final 1X Reagent necessary for the experiments of the day. Stability of the Final 1X Reagent, as diluted from Buffer 4 has not been evaluated.

MATERIAL REQUIRED BUT NOT PROVIDED

Specimen for testing: Whole blood or bone marrow in anti-coagulant tube. The blood specimen should be used as soon as possible in order to preserve epitopes and cellular structures (within 24h). Depending on the application, older specimens can be analysed.

PBMC and cell lines, either fresh or frozen, must be prepared according to good laboratory practices and then treated according to the specific procedure indicated on page 2.

Equipment:

- Pipettors to deliver from 1 to 1000 µL
- 5 mL plastic tubes
- Timer
- Water bath, set at 37°C.
- Automatic agitator (Vortex type)
- Fluorochrome-conjugated antibodies against intracellular epitopes and surface molecules
- Centrifuge, variable speed
- Flow Cytometer

PROCEDURE FOR WHOLE BLOOD OR TOTAL BONE MARROW

1. Pipet 100 µL of specimen into the bottom of each appropriately labeled tube. Avoid putting specimen on the side surface of the tube where it will not be appropriately treated.
2. If necessary, add activators or inhibitors of signaling pathways; mix well and incubate for the recommended time at the recommended temperature (usually 37°C). Water bath is preferable to dry incubator.
3. Pipet 50 µL of Buffer 1 (Fixative Reagent) to each tube, vortex immediately (1 – 2 seconds) and incubate for 10 min at room temperature (18 – 25°C).
4. Vortex briefly the fixed specimen and add 1 mL of Buffer 2 (Permeabilizing Reagent) to each tube, vortex immediately (1 – 2 seconds) and incubate for exactly 5 min. at 37°C in a water bath.
5. Immediately centrifuge the cells at 300 x g for 5 minutes and completely discard the supernatant by aspiration. (Inverting the tubes is not appropriate).
6. Add immediately to each tube 100 µL of Buffer 3 (Staining Reagent) and then add the fluorochrome-conjugated antibodies against phospho-epitopes, intracellular epitopes and surface molecules (preferably, the antibodies are pre-mixed

BECKMAN COULTER

into the Buffer 3 and both volumes are added altogether on the cell pellet).

7. Vortex immediately (1−2 seconds) and incubate for 15 – 30 min at room temperature protected from light.
8. Add 3 mL of the Final 1X Reagent (Wash reagent) prepared from Buffer 4 (20X concentrated solution) to each tube, vortex immediately (1−2 seconds), centrifuge the cells at 300 x g for 5 minutes and completely discard the supernatant by aspiration.
9. Resuspend the cell pellet in 0.5 mL of the Final 1X Reagent. Sample is now ready for analysis on a flow cytometer.

SPECIFIC PROCEDURE FOR PBMC OR CELLS

IMPORTANT: Wash the cells, centrifuge, and resuspend the cell pellet with pure FCS or FBS (Fetal Calf/ Bovine Serum) 2.10^5 to 1.10^6 Cells/test.

1. Dispense 100 µL of this cell suspension into the bottom of each appropriately labeled tube. Avoid putting some sample on the side surface of the tube where it will not be appropriately treated.
2. If necessary, add activators or inhibitors of signaling pathways; mix well and incubate the recommended time at the recommended temperature (usually 37°C). Water bath is preferable to dry incubator.
3. Pipet 25 µL of Buffer 1 (Fixative Reagent) to each tube, vortex immediately (1−2 seconds) and incubate for 10 min at room temperature (18 – 25°C).
4. Vortex again the fixed cells and add 0.5 mL of FCS or FBS. Add 0.5 mL of Buffer 2 (Permeabilizing Reagent) to each tube, vortex immediately (1−2 seconds) and incubate for exactly 5 min. at 37°C in a water bath.
5. Immediately centrifuge the cells at 300 x g for 5 minutes and completely discard the supernatant by aspiration. (Inverting the tubes is not appropriate).
6. Add immediately to each tube 50 µL of Buffer 3 (Staining Reagent) and then add the fluorochrome-conjugated antibodies against phospho-epitopes, intracellular epitopes and surface molecules (preferably, the antibodies are pre-mixed into the Buffer 3 and both volumes are added altogether on the cell pellet).
7. Vortex immediately (1−2 seconds) and incubate for 15 – 30 min at room temperature protected from light.
8. Add 3 mL of the Final 1X Reagent (Wash reagent) prepared from Buffer 4 (20X concentrated solution) to each tube, vortex immediately (1−2 seconds), centrifuge the cells at 300 x g for 5 minutes and completely discard the supernatant by aspiration.
9. Resuspend the cell pellet in 0.5 mL of the Final 1X Reagent.

SPECIAL NOTES

1. Volume of wash buffer: Since the "PBMC or Cells" procedure recommends using only half of the recommended volumes of Buffers 1, 2, and 3, we added some extra Buffer 4 to offer the possibility to users performing the "PBMC or Cells" procedure to run **150 tests**.
2. All conjugated antibodies, for intra- and extra-cellular staining, must be titrated for optimal results.
3. Fixative and detergent concentrations could be detrimental to surface epitopes. In this case, it is recommendded to either choose another antibody clone or another marker or to add antibodies to the specimen before the fixation step, and thus pre-incubate them with the sample either before or during the activation/ inhibition step (after verifying that this pre-incubation step does not interfere with the signaling pathway).
4. 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.
5. When possible, it is useful (and recommended) to batch the tests:
   a. Multiple experiments on the same specimen will benefit from a batch activation and fixation (upscale sample volume and Buffer 1 as necessary, then distribute e.g. 155 µL (100 µL blood + 5 µL activator/inhibitor + 50 µL Buffer 1) into the test tubes during the 10 min incubation time (or at the 10 min time point if the tubes already contain the 1 mL of Buffer 2).
   b. Next step can also be batched: upscale the volume of Buffer 2 and add it on the batch of fixated sample, incubate 5 min. at 37°C (insure homogeneity), then centrifuge and resuspend the batch pellet in Buffer 3, then distribute into multiple tubes containing various antibody combinations.
   c. Multiple experiments on different specimens but with the same antibody cocktails will benefit from a batch preparation of the Buffer 3 + antibodies.
6. Ultimately, when the procedure is optimized, reproducible in the lab, and when the signal ratio of activated versus non-activated cells is at least higher than 3, the mix of activated and non-activated cells into one single tube can be implemented. It should occur at the end of the fixation step. It will insure that both samples are treated exactly the same during all subsequent steps, and reinforce the accuracy of the results.

   The mix can be done between 7 and 10 min. after starting the fixation: Vortex again each sample immediately before mixing. Then proceed with the procedure (step 4), maintaining the ratio of 1 mL Buffer 2 per 100 µL whole blood sample (or 500 µL Buffer 2 per 100 µL PBMC or Cells).

EXAMPLE of BATCH and MIX: titration of a conjugate on whole blood (6 titration points):

1. Pipet 2x300 µL of whole blood sample in 2 test tubes, add an activator in the second test tube and incubate both tubes at 37°C.
2. Add 150 µL of Buffer 1 in both tubes, vortex and incubate 7 min (see special note n°6).
3. MIX: Transfer all 450 µL (300 µL blood+150 µL Buffer 1) of tube 1 and 450 µL (+ activator volume) of tube 2 into a > 7 mL tube and incubate until the 10 minutes end point of fixation.
4. Add 6 mL of Buffer 2, mix well and incubate 5 min. at 37°C, centrifuge, and aspirate supernatant.
5. Resuspend the pellet into 300 µL of Buffer 3 and distribute 6x50 µL into 6 test tubes each containing 50 µL of Buffer 3 plus the various volumes of the conjugate.
6. Incubate 15-30 min. and proceed with the normal procedure steps 8-9.

TRADEMARKS

Manufactured by:
Immunotech SAS
a Beckman Coulter Company
130, avenue de Lattre de Tassigny
B.P. 177 - 13276 Marseille Cedex 9
France

For additional information in the USA, call 800-526-7694.
Outside the USA, contact your local Beckman Coulter representative.

www.beckmancoulter.com

Printed in France.
Made in France.