Fixation and Permeabilization Buffer Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Fixation and Permeabilization Buffer Kit (50 tests)

Catalog No. FIXPERM01

Sample insert for reference use only

Principle of the Assay

The Fixation and Permeabilization Buffer Kit is designed for performing FACS and/or ICC analysis.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all samples prior to starting the procedure.
- The Fixation Buffer contains 4% paraformaldehyde.
- The Permeabilization Buffer contains Triton™ X-100.
- The kit should not be used beyond the expiration date.

Reagents

- **Fixation Buffer (1x)**: A solution to fix cells prior to permeabilization (25 ml, 1 bottle).
- **Permeabilization Buffer (1x)**: A surfactant solution to permeabilize cells prior to staining (25 ml, 1 bottle).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Fixation Buffer (1x) and Permeabilization Buffer (1x) at 2-8°C.

Other Supplies Required

- Flow Cytometer
- Fluorescent Microscope
- Pipettes (1-20 µl, 20-200 µl, and 200-1000 µl)
- 1x PBS, pH 7.4
- Deionized or distilled reagent grade water
- 1x DAPI
- Antifade Mountant

- FACS Buffer (10x): *Available for order from Assaypro, catalog no.* ***FACS***
- Blocking Buffer (10x): *Available for order from Assaypro, catalog no.* ***BB***

Sample Preparation

- Cells, at about 80% confluence, were collected from 8-10 Petri Dishes/T-75 flasks (2-10 million cells/flask) into conical centrifuge tubes.
- Centrifuge cells at 1300 rpm for 5 minutes at 20°C. Aspirate the supernatant. Wash the pellet once with 10 ml of 1x PBS, pH 7.4 and proceed immediately with the FACS procedure.

FACS Procedure

- Prepare all reagents and samples as instructed. Keep all reagents at 4°C. The procedure is performed at room temperature (20-25°C). Make sure to resuspend the pellet thoroughly every time after centrifugation.
- Centrifugation takes place for 5 minutes at 1300 rpm and 20°C. **Centrifuge and aspirate after each step**.
- Add 2 ml of Fixation Buffer to the prepared sample in the conical centrifuge tubes. Resuspend and incubate for 20 minutes.
- **(Omit this step if you plan to do surface staining)** Wash the pellet two times with 7 ml of FACS Buffer.
- **(Omit this step if you plan to do surface staining)** Add 2 ml of Permeabilization Buffer to the pellet. Resuspend and incubate for 20 minutes.
- Wash the pellet two times with 7 ml of FACS Buffer.
- Add 2 ml of Blocking Buffer to the pellet. Resuspend and incubate for 1 hour.
- Resuspend the pellet in 3 ml of FACS Buffer. Mix the cell suspension thoroughly to make sure there are no visible cell clumps. Aliquot 100 µl of the cell suspension into 5 ml tubes. Add fluorescent conjugated antibody at set concentration (1-2 µg/tube for APC, RPE, and FITC conjugates or 10 µg/tube for PerCP conjugate). Incubate in a dark environment for 45 minutes. Agitate the tube every 5 minutes during incubation to ensure proper mixing or place the tube on a shaker for the entirety of the incubation period.
- Wash the pellet two times with 2 ml of FACS Buffer and centrifuge the tube. Leave 250 µl of supernatant in the tube and aspirate the rest. Add 1 ml of FACS Buffer to resuspend and analyze on a flow cytometer.

ICC Staining/Corning® Falcon® CultureSlides

- Keep all reagents at 4°C. Make sure cells are at the proper density before proceeding to the next step. **Aspirate after each step.**
- Aspirate media. Add 0.5 ml of 1x PBS to each chamber.
- Add 0.4 ml of Fixation Buffer and incubate for 20 minutes at room temperature. Cover wells during incubation.
- Add 0.5 ml of 1x PBS and incubate for 5 minutes at room temperature. Repeat once.
- Add 0.4 ml of Permeabilization Buffer and incubate for 20 minutes at room temperature.
- Add 0.5 ml of 1x PBS and incubate for 5 minutes at room temperature. Repeat once.
- Add 0.4 ml of Blocking Buffer and incubate for 1 hour at room temperature.
- Add 0.5 ml of 1x PBS and incubate for 5 minutes at room temperature. Repeat once.
- Add fluorescent conjugated antibody to a final volume of 400 µl. Incubate overnight at 4°C.
- Add 0.5 ml of 1x PBS and incubate for 5 minutes at room temperature. Repeat once.
- Stain with 1x DAPI (400 µl/well). Incubate for 4 minutes at room temperature.
- Add 0.5 ml of 1x PBS and incubate for 5 minutes at room temperature. Repeat once.
- Add 25 µl of antifade mountant to each well. Cover with Corning® glass slide cover and incubate overnight at room temperature in a dark environment. **Do NOT aspirate**.
- Analyze using a Fluorescent Microscope.

Version 1.2