AssayLite™

Human BRD1 FACS Kit

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This product is manufactured under patented technology by Assaypro LLC

US Patent No. 9,945,847

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

Thank you for choosing Assaypro.

Procedure Summary

Step 1. Add 2 ml of Fixation Buffer to resuspend the pellet.
Incubate 20 minutes.

Step 2. (Omit this step if you plan to do surface staining)
Wash, then add 2 ml of Permeabilization Buffer to resuspend the pellet.
Incubate 20 minutes

Step 3. Wash, then add 2 ml of Blocking Buffer to resuspend the pellet.
Incubate 1 hour.

Step 4. Stain cell with Fluorescent Antibody or Fluorescent Isotype Control.
Incubate 45 minutes.

Step 5. Wash, then add 1 ml of FACS Buffer to resuspend the pellet.
Analyze on flow cytometer.

AssayLite™ Human BRD1 FACS Kit (25 tests)

Catalog No. FACS33128A

Sample insert for reference use only

Principle of the Assay

The AssayLite™ Human BRD1 FACS (Fluorescence-activated Cell Sorter) Kit is designed for performing flow cytometry analysis to human BRD1.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents (blocking Buffer, FACS buffer, fluorescent antibody, and fluorescent isotype control) as instructed, prior to starting the procedure.
- 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

- **Blocking Buffer Concentrate (10x)**: A 10-fold concentrated buffered solution for blocking (1 ml).
- **FACS Buffer Concentrate (10x)**: A 10-fold concentrated buffered solution for FACS (30 ml).
- **Fixation Buffer (1x)**: A solution to fix cells prior to permeabilization (9 ml).
- **Permeabilization Buffer (1x)**: A surfactant solution to permeabilize cells prior to staining (9 ml).
- **Human BRD1 Antibody [APC Conjugate] (1x):** Lyophilized, 2 vials.
- **Fluorescent Isotype Control [APC Conjugate] (1x):** Lyophilized, 2 vials.

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store FACS Buffer Concentrate (10x), Blocking Buffer Concentrate (10x), Fixation Buffer (1x), and Permeabilization Buffer (1x) at 2-8°C.

- Store Fluorescent Antibody and Fluorescent Isotype Control in a dark place at 2-8°C. Do not freeze.

Other Supplies Required

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

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, 7.4 and proceed immediately with the FACS procedure.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to 4°C before use.
- **Blocking Buffer Concentrate (10x)**: Dilute the Blocking Buffer Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
- **FACS Buffer Concentrate (10x)**: Dilute the FACS Buffer Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
- **Human BRD1 Fluorescent Antibody [APC Conjugate] (1x):** Reconstitute the antibody with 0.3 ml of reagent grade water to produce a 1x solution. Allow the antibody to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be stored at 2-8°C and used within 30 days. Do not freeze.
- **Fluorescent Isotype Control [APC Conjugate] (1x):** Reconstitute the control with 0.3 ml of reagent grade water to produce a 1x solution. Allow the control to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be stored at 2-8°C and used within 30 days. Do not freeze.

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 7ml 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 7ml 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 20 µl of Human BRD1 Fluorescent Antibody or Fluorescent Isotype Control to each tube to stain the cells. 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.

Typical Data

- FACS analysis of A549 (human adenocarcinoma) cells was performed in house (Figure 1). The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

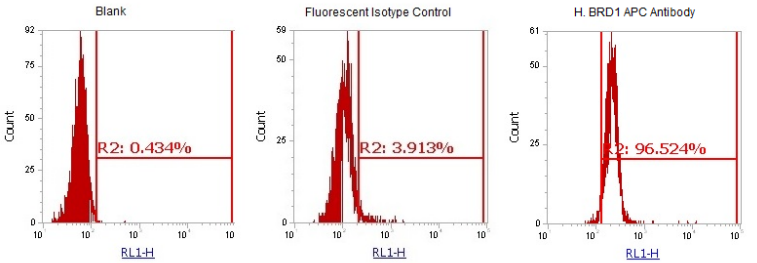

Figure 1. FACS analysis of A549 (human adenocarcinoma) cells using this kit.

Version 1.0