

# Monoclonal Anti-human Cripto-1-Phycoerythrin

Catalog Number: FAB2772P

Lot Number: LRW03

100 Tests

## Reagents Provided

**Phycoerythrin (PE)-conjugated mouse monoclonal anti-human Cripto-1:** Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

**Clone #:** 89633

**Isotype:** mouse IgG<sub>1</sub>

## Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

## Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

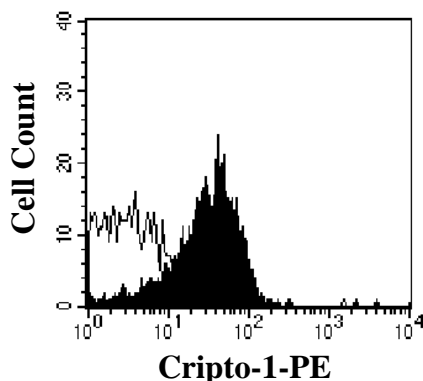
Designed to quantitatively determine the percentage of cells bearing Cripto-1 within a population and qualitatively determine the density of Cripto-1 on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells bearing Cripto-1. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing Cripto-1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Cripto-1. Cell surface expression of Cripto-1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

## Reagent Preparation

**PE-conjugated mouse anti-human Cripto-1:** Use as is; no preparation necessary.



Human peripheral blood monocytes stained with PE-conjugated anti-Cripto-1 (Catalog # FAB2772P, filled histogram) or isotype control (Catalog # IC002P, open histogram).

## Sample Preparation

**Peripheral blood cells:** Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

**Cell Cultures:** Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

## Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated anti-Cripto-1 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted anti-Cripto-1 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
- 7) As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgG<sub>1</sub> antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

**R&D Systems, Inc.**  
**1-800-343-7475**

## Background Information

Cripto-1, also known as CFC-2 or TDGF (teratocarcinoma-derived growth factor), is a member of the epidermal growth factor-CriptoFRL1Cryptic (EGF-CFC) family of signaling proteins that function in various developmental processes and in cancer. It exists both as a secreted and as a GPI-anchored membrane protein and acts as a cofactor to modulate the activities of TGF- $\beta$  family members (1, 2). Cripto-1 is highly expressed in embryonic stem cells (ESCs) (3) and is involved in neural induction from ESCs (4). Cripto-1 is also expressed in human peripheral blood monocytes (5). Cripto-1 is over-expressed in many types of cancers (2, 6) and has been considered a target for cancer immunotherapy.

## References

1. Adamson, E.D. *et al.* (2002) *J. Cell. Physiol.* **190** :267.
2. Saloman, D.S. *et al.* (2000) *Endocr. Relat .Cancer* **7**:199.
3. Bhattacharya, B. *et al.* (2004) *Blood* **103**:2956.
4. Parish, C.L. *et al.* (2005) *Stem Cells* **23**:471.
5. R&D Systems in house data.
6. Minchiotti, G. *et al.* (2002) *Gene* **287**:33.

**Warning:** Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.