

## Reagents Provided

**Phycoerythrin (PE)-conjugated mouse monoclonal anti-human CRHR-1:** Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 343919

**Isotype:** mouse IgG<sub>2A</sub>

## Reagents Not Provided

- 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

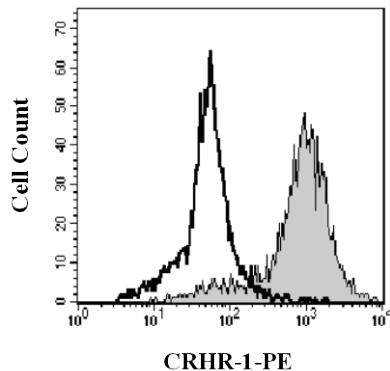
Designed to quantitatively determine the percentage of cells bearing CRHR-1 within a population and qualitatively determine the density of CRHR-1 on cell surfaces by flow cytometry.

## Principle of the Test

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

## Reagent Preparation

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



CRHR-1/Baf3 transfectants were stained with PE-conjugated anti-human CRHR-1 (Catalog # FAB3930P, filled histogram) or PE-conjugated isotype control (Catalog # IC003P, 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 (up to 1 x 10<sup>6</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated CRHR-1 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CRHR-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>2A</sub> antibody.

This procedure may need modification, depending upon final utilization.

## Background Information

Corticotropin-releasing hormone receptor 1 (CRHR-1) is a member of the Class B G protein-coupled receptor family that mediates the action of hypothalamic and peripheral CRH. It is the key regulator of the stress response in the hypothalamic-pituitary adrenal axis and an important functional mediator of the endocrine, cardiovascular, gastrointestinal, and immune systems. The other major endogenous ligand for CRHR-1 is urocortin 1. CRHR-1 is highly expressed in the cerebral cortex, striatum, amygdala, and cerebellum in rodents (1). In humans, transcripts are detected in cortex, brainstem, and pituitary, but not in placenta and peripheral blood lymphocytes (2). It is also expressed in human adrenal cortex and adrenal tumor cell line NCI-H295R (3), non-pregnant and pregnant myometrium (4), and weakly in placental tissues (5).

## References

1. Castro, M.G. *et al.* (1996) *J. Neuroendocrinol.* **8**:521.
2. Vita, N. *et al.* (1993) *FEBS* **335**:1.
3. Willenberg, H.S. *et al.* (2006) *Neuroendocrinol.* **82**:274.
4. Rodriguez-Linares, B. *et al.* 1998, *J. Endocrinol.* **156**:15.
5. Sehringer, B. *et al.* 2004, *J. Mol. Endocrinol.* **32**:339.

**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.