

# Polycional Anti-human PILR-α-Phycoerythrin Catalog Number: FAB6484P

Lot Number: ACHH01

100 Tests

### **Reagents Provided**

Phycoerythrin (PE)-conjugated sheep polyclonal anti-human PILR- $\alpha$ : Supplied as 10  $\mu$ g of antibody in 1.0 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Isotype: sheep IgG

#### **Reagents Not Provided**

• Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

#### Storage

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

#### **Intended Use**

Designed to quantitatively determine the percentage of cells bearing PILR- $\alpha$  within a population and qualitatively determine the density of PILR- $\alpha$  on cell surfaces by flow cytometry.

#### **Product Description**

This antibody was produced in sheep immunized with purified, NS0-derived, recombinant human PILR- $\alpha$  (rhPILR- $\alpha$ ; aa 20-196; Accession # Q9UKJ1) extracellular domain. Human PILR- $\alpha$  specific IgG was purified by human PILR- $\alpha$  affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of PILR- $\alpha$  is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565-605 nm.



Human peripheral blood granulocytes were stained with PE-conjugated anti-human PILR- $\alpha$  (Catalog # FAB6484P, filled histogram) or PE-conjugated control antibody (Catalog # IC016P, open histogram).

## Background Information

PILR- $\alpha$  (paired immunoglobulin-like, type 2 receptor alpha) is a 44-50 kDa type I transmembrane paired receptor glycoprotein that belongs to the Ig superfamily. It is expressed by monocytes, macrophages, CD14<sup>+</sup>CD1a<sup>-</sup> dendritic cells, and retinal pigment cells, and is known to bind to CD99 and PANP. PILR- $\alpha$  acts as a receptor for HSV and serves as a negative immunomodulator that contains an ITIM. Mature human PILR- $\alpha$  is 284 amino acids (aa) in length. It contains one V-type Ig-like domain in its extracellular region (aa 32-150), and two ITIMs in its cytoplasmic domain (aa 267-272 and 296-301). There are multiple potential splice variants. One is a transmembrane isoform that possesses a 35 aa substitution for aa 264-303, while others are soluble, and show a deletion of aa 152-224 that may be coupled to the 35 aa substitution noted above, or simply exhibit a 24 aa substitution for aa 152-303. Over aa 20-196, human PILR- $\alpha$ shares only 42% aa identity with mouse PILR- $\alpha$ , and 89% aa identity with human PILR-β.

#### **Flow Cytometry Validation**

This antibody has been tested for flow cytometry using human peripheral blood granulocytes.

- Cells may be Fc-blocked with 1 μg of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- 2. After blocking, 10  $\mu L$  of conjugated antibody was added to up to 1 x 10<sup>6</sup> cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
- 4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PE-labeled sheep IgG antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

**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.