

## Reagents Provided

### Alexa Fluor<sup>®</sup> 488-conjugated sheep polyclonal anti-human PILR- $\alpha$ :

Supplied as 10  $\mu$ g of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Isotype:** sheep IgG

## Reagents Not Provided

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

## Storage

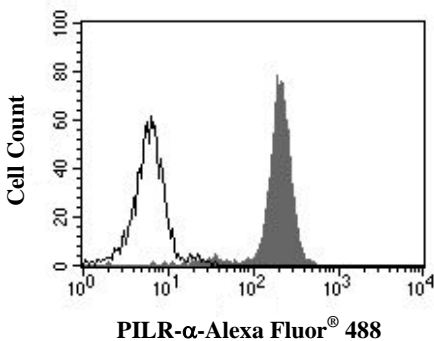
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## 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 Alexa Fluor<sup>®</sup> 488 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 515 - 545 nm.



Human peripheral blood granulocytes were stained with Alexa Fluor<sup>®</sup> 488-conjugated anti-human PILR- $\alpha$  (Catalog # FAB6484G, filled histogram) or Alexa Fluor<sup>®</sup> 488-conjugated control antibody (Catalog # IC016G, open histogram).

## Legal

This product is provided under an agreement between Life Technologies Corporation and R&D Systems, Inc, and the manufacture, use, sale or import of this product is subject to one or more US patents and corresponding non-US equivalents, owned by Life Technologies Corporation and its affiliates. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components (1) in manufacturing; (2) to provide a service, information, or data to an unaffiliated third party for payment; (3) for therapeutic, diagnostic or prophylactic purposes; (4) to resell, sell, or otherwise transfer this product or its components to any third party, or for any other commercial purpose. Life Technologies Corporation will not assert a claim against the buyer of the infringement of the above patents based on the manufacture, use or sale of a commercial product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, Cell Analysis Business Unit, Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

## 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- $\beta$ .

## Flow Cytometry Validation

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

- Cells may be Fc-blocked with 1  $\mu$ g of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 5  $\mu$ 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).
- 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 Alexa Fluor<sup>®</sup> 488-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.