

## Reagents Provided

**Allophycocyanin (APC)-conjugated sheep polyclonal anti-mouse Gi24/Dies1/VISTA:** Supplied as 10 µg of antibody in 1 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

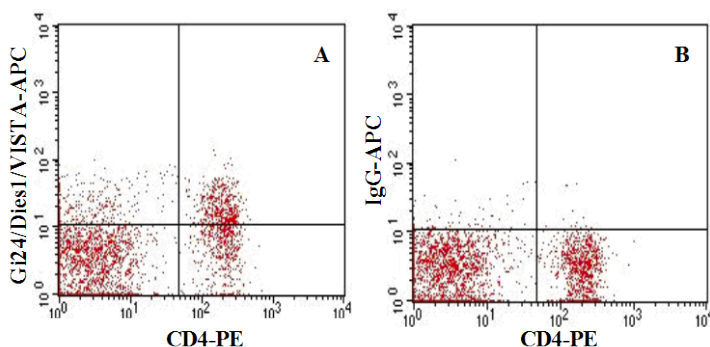
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing Gi24/Dies1/VISTA within a population and qualitatively determine the density of Gi24/Dies1/VISTA on cell surfaces by flow cytometry.

## Product Description

This antibody was produced in sheep immunized with purified NS0-derived recombinant mouse Gi24/Dies1/VISTA (Gi24/Dies1/VISTA; Accession # Q9D659). Mouse Gi24/Dies1/VISTA specific IgG was purified by mouse Gi24/Dies1/VISTA affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of Gi24/Dies1/VISTA is determined by flow cytometry using 620-650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660-670 nm.



Mouse splenocytes were stained with PE-conjugated anti-mouse CD4 (Catalog # FAB554P) and either A) APC-conjugated anti-mouse Gi24/Dies1/VISTA (Catalog # FAB7005A) or B) APC-conjugated isotype control (Catalog # IC016A).

## Background Information

Platelet Receptor Gi24 (also known as Dies1 [Differentiation of ESC1], SISP1, and C10orf54) is a 5565 kDa member of the Ig superfamily. It is a transmembrane molecule expressed in bone, on embryonic stem cells (ESCs), and on tumor cell surfaces. On ESCs, Gi24 appears to positively interact with BMP-4, potentiating BMP signaling and the transition from an undifferentiated to a differentiated state. On tumor cells, Gi24 both promotes MT1-MMP expression and activity, and serves as a substrate for MT1-MMP. This increases the potential for cell motility. VISTA has been described as an inhibitory B7 family ligand.<sup>1</sup> Mature mouse Gi24 is a 277 amino acid (aa) type I transmembrane glycoprotein. Mouse Gi24/Dies1 shares 78% and 70% aa sequence identity with rat and human Gi24, respectively.

## References

- Wang, L. *et al.* (2011) *J. Exp. Med.* **208**:577.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

- Cells may be Fc-blocked with 1 µg of mouse IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL 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 Mouse Lyse Buffer (Catalog # FC003).
- 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 APC-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.