Polyclonal Anti-GFP-Phycoerythrin

Catalog Number: IC4240P
Lot Number: ABZH01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-jellyfish GFP: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: goat IgG

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

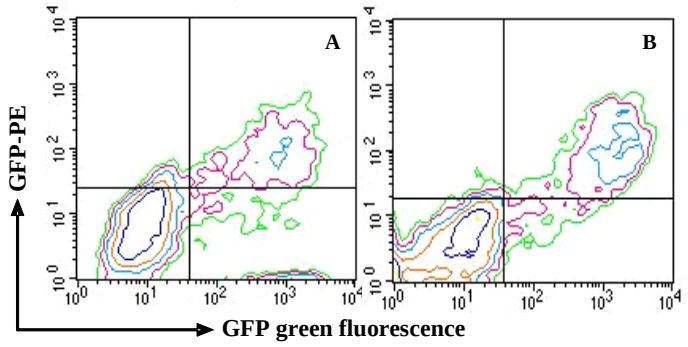
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing GFP within a population and qualitatively determine the density of intracellular GFP by flow cytometry.

Product Description

Produced in goats immunized with purified, *E. coli*-derived, recombinant GFP (aa 2 - 238; Accession # AAB65663). GFP specific IgG was purified by GFP affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Intracellular expression of GFP 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.



Contour plots show PE-conjugated anti-GFP (Catalog # IC4240P) staining versus GFP green fluorescence in HEK293 cells transiently expressing GFP wild type (A) or EGFP (B).

Background Information

The Green Fluorescent Protein (GFP), originally identified and isolated from the jellyfish *Aequorea victoria*, is a universally used fluorescent indicator, utilized as a reporter for gene expression, a cell lineage tracer, and for measurements of protein-protein interactions. GFP and chimeric proteins fused to GFP have been expressed in virtually all types of cell and are used as non-invasive fluorescent markers in living cells and organisms. GFP is approximately 27 kD and 238 amino acids and emits green light when excited with blue or UV light (emission peak at 509 nm and excitation peak at 395 nm). Given its widespread use and potential applications, several GFP mutants have been developed, including enhanced GFP (EGFP), commonly used in mammalian cells.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see Reagents Not Provided).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Up to 1 x 10^6 cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 µL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PE-labeled goat IgG antibody. This procedure may need to be modified, depending on 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.

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