Product Information

Anti-Green Fluorescent Protein (GFP) antibody, Mouse monoclonal

Clone GSN149, purified from hybridoma cell culture

G1546

Product Description

Monoclonal Anti-Green Fluorescent Protein (GFP) (mouse IgG1 isotype) is derived from the hybridoma GSN149 produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to a fragment of the Green Fluorescent Protein from jellyfish *Aequorea victoria*. The isotype is determined using a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Cat. Nos. G1546, ISO2.

Monoclonal Anti-Green Fluorescent Protein reacts specifically with GFP-fusion proteins. Applications include ELISA and immunoblotting.

The spontaneously fluorescent protein GFP is a unique tool in cellular and molecular biology research.[1] In the jellyfish *A. victoria*, GFP transduces the excitation energy resulting from emission of blue light by the photoprotein aqueorin, and reemits it as green light.[2,3] Cloning revealed GFP as a 27 kDa protein (238 amino acids) that is capable of producing a strong green fluorescence without the need for a substrate. It absorbs light maximally at 395 nm and emits a bright green fluorescence with a peak at 509 nm.[1] The GFP chromophore is formed through cyclization and oxidation of an internal tripeptide motif (Ser,[65] Gly,[69] and Tyr[66]).[4]

The DNA sequence of GFP can be inserted as a fusion protein with any gene of interest so that it is produced concomitantly with the expression of the gene of interest. Thus, the fusion protein emits green light and can then be visualized. Altogether, the above findings led to the development of a large number of applications using GFP as a fusion protein. These include protein detection and localization in living cells, as well as gene expression monitoring in prokaryote and eukaryotes.[2,5-6] More recently, cyan and yellow variants of the green fluorescence protein were developed.[7-9]

The different spectral properties of the variants provide a powerful approach for tracking the fate of two proteins simultaneously in the same or in different intracellular compartments, and for studying protein-protein interactions in living cells.[9,10] Antibodies to GFP may be useful in various immunoassays, to identify the successful expression of a GFP fusion protein, to correlate levels of GFP protein expression with fluorescence intensity, and for immunoprecipitation of GFP fusion proteins.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: ~2 mg/mL

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For extended storage, freeze at –20 °C in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 1-2 µg/mL is recommended using extracts of cells expressing GFP fusion proteins.

At least 6-9 ng of purified GFP can detected with 2 µg/mL of the antibody by immunoblotting.

Note: In order to obtain best results in various techniques and preparations, it is recommended to determine optimal working dilutions by titration.

Procedure for Immunoblotting

Note: Perform the whole procedure at room temperature.

1. Separate GFP tagged proteins from sample lysates using a standard sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5-20 µg total lysate protein per lane.

   Note: The amount of lysate to be loaded depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of PBS containing 5% non-fat dry milk (Dulbecco's Phosphate Buffered Saline, Cat. No. D8537; Nonfat-Dried Milk, Cat. No. M7409) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Cat. No. P3563.
5. Incubate the membrane with Monoclonal Anti-GFP as the primary antibody in PBS containing 1% BSA, with agitation for 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween® 20 plus 1% BSA.
7. Incubate the membrane with Anti-Mouse IgG (Fab specific)–Peroxidase, Cat. No. A2304, as the secondary antibody at the recommended concentration in PBS containing 0.05% Tween® 20 and 1% BSA. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween® 20.
9. Treat the membrane with a peroxidase substrate.

References

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