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Product Information

Anti-Green Fluorescent Protein (GFP)
N-terminal antibody, Mouse monoclonal
clone GSN24, purified from hybridoma cell culture

Product Number **G6795**

Product Description

Anti-Green Fluorescent Protein (GFP), N-terminal antibody, Mouse monoclonal (mouse IgG1 isotype) is derived from the hybridoma GSN24 produced by the fusion of mouse myeloma cells (NS1 cells) and splenocytes from BALB/c mice. The mice are immunized with a peptide corresponding to a fragment of the Green Fluorescent Protein from the jellyfish, *Aequorea victoria,* conjugated to KLH through an N-terminal added cysteine residue. The isotype is determined using a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2.

Anti-Green Fluorescent Protein (GFP), N-terminal antibody, Mouse monoclonal reacts specifically with GFP fusion proteins and is compatible with 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 of the photoprotein aequorin, and reemits it as green light.[2,3] Cloning revealed GFP to be a 27 kDa protein (238 amino acids) that is capable of producing a strong green fluorescence without the need for a substrate. It has an absorbance maximum 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 with any gene of interest and a fusion protein 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 for GFP fusion proteins. These include protein detection and localization in living cells, and gene expression monitoring in prokaryotes and eukaryotes.[2,5,6]

In addition, cyan and yellow variants of Green Fluorescence Protein have been 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 immuno-techniques including successful identification of expressed GFP fusion proteins and correlation of GFP expression levels with fluorescent intensity.

Reagents

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

Antibody Concentration: ~2.0 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 continuous use, store at 2–8 °C for up to one month. For extended storage, store at –20 °C in working aliquots. Repeated freezing and thawing is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Solutions at working dilution should be discarded if not used within 12 hours.

Procedure

Immunoblotting Procedure – Perform the entire procedure at room temperature.

1. Separate GFP tagged proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5–20 μg of 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 SDS-PAGE gel to a nitrocellulose membrane.
3. Block the membrane using a solution of PBS containing 5% non-fat dry milk (PBS, Product Code D8537; nonfat-dried milk, Product Code M7409) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (Product Code P3563).
5. Incubate the membrane with anti-GFP, N-terminal, antibody 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 and 1% BSA.
7. Incubate the membrane with anti-mouse IgG (Fab specific)-peroxidase conjugate (Product Code 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.

Product Profile

Immunoblotting: a working concentration of 1-2 μg/mL is determined by using GFP fusion proteins expressed in extracts of transfected cells.

At least 3-6 ng of purified GFP can be 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.

References

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