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ProductInformation

Anti-GFP, N-Terminal
Developed in Rabbit, Affinity Isolated Antibody

Product Number: **G 1544**

Product Description

Anti-GFP, N-Terminal is developed in rabbit using a peptide corresponding to amino acids 3-17 of the Green Fluorescent Protein (GFP) from jellyfish *Aequorea victoria,* conjugated to maleimide-activated KLH through an N-terminal added cysteine residue as immunogen. The antibody is affinity-purified on the immunizing peptide immobilized on agarose.

Anti-GFP, N-Terminal reacts specifically with GFP-fusion proteins. Applications include immunoblotting and immunoprecipitation. Staining of GFP-fusion proteins by immunoblotting is specifically inhibited by the GFP immunizing peptide.

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 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. The above findings have led to the development of a large number of applications using GFP as fusion protein, including protein detection and localization in living cells, as well as gene expression monitoring in prokaryotes and eukaryotes.[2, 5, 6] Cyan and yellow variants of the 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 different intracellular compartments, and for studying protein-protein interactions in living cells.[9, 10]

Antibodies to GFP may be useful in various immunotechniques to identify the expression of a GFP fusion protein, to correlate levels of GFP protein expression with fluorescence intensity and for immunoprecipitation of GFP fusion proteins.

Reagent

Anti-GFP, N-Terminal is provided in 0.01 M phosphate buffered saline pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Antibody Concentration: Approx. 1.0 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS 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 freeze in working aliquots. Repeated freezing and thawing is not recommended. 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.

Procedures

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 (PBS, Product No. D 8537; non-fat dry milk, Product No. M 7409) for at least 60 min.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20 (Product No. P 3563).
5. Incubate the membrane with anti-GFP (N-ter) antibody as the primary antibody in PBS containing 0.05 % Tween 20, with agitation for 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20.
7. Incubate the membrane with anti-rabbit IgG, peroxidase conjugate (Product No. A 0545) as the secondary antibody at the recommended concentration in PBS containing 0.05% Tween 20. 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.

Procedure for Immunoprecipitation

1. Centrifuge 40 µl of a 1:1 suspension of protein A-agarose beads (Product No. P 3476) for 1 minute at 2000 g, and then wash twice with 1 ml RIPA buffer (50 mM Tris Base, 0.25% w/v Deoxycholate, 1% NP40, 150 mM NaCl, 1mM EDTA, pH 7.4) at 4 ºC.
2. Add anti-GFP (N-ter) antibody diluted in PBS, and incubate by swinging head-over-tail for 1 hour at room temperature.
3. Centrifuge for 1 minute at 12,000g, and wash twice with 1 ml RIPA at 4 ºC by spinning.
4. Add 0.1-1.0 ml of cell extract containing GFP tagged protein to the beads (see Note), and incubate from 2 hours to overnight at 4 ºC, while swinging head-over tail.
   Note: The amount of cell extract depends on the level of expression of the tagged protein and the specific application.
5. Spin down beads; remove supernatant.
6. Wash beads four times with 1ml RIPA buffer and once with PBS by vortex and short spin.
7. Resuspend the pellet in 25 µl of 2XSDS-PAGE sample buffer. Boil sample for 5 minutes and spin down. The sample is ready to be loaded on an SDS-PAGE gel.

Product Profile

A working concentration of 0.25-0.5 µg/ml is determined by immunoblotting of GFP fusion proteins expressed in cell extracts of transfected cells.
At least 3-6 ng of purified GFP can be detected with 0.25 µg/ml of the antibody by immunoblotting.

1.0-2.5 µg of the antibody can immunoprecipitate a GFP fusion protein from transfected mammalian cell lysates.

Note: In order to obtain the best results and assay sensitivity in various techniques and preparations, we recommend determining the optimal working concentrations by titration.

References

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