



Product Specification Sheet

Monoclonal Anti-Green Fluorescent Protein (GFP)

Cat. GFP12-M	Mouse Monoclonal Anti-GFP IgG	SIZE: 100 ug
Cat. GFP11-C	Recombinant purified GFP protein for WB control	SIZE: 100 ul

Recombinant DNA technology allows the addition of short pieces of well-defined tags, "peptides" or proteins at the amino or c-terminus of target genes, which can provide 'affinity handles' designed to bind specific matrices. Therefore, tags enables a selective identification and purification of the protein of interest. The addition of a green fluorescent protein (GFP) tag to a given gene, creates a stable fusion product that does not appear to interfere with the bioactivity of the protein, or with the biodistribution of the GFP tagged product. GFP is a 27 kD (238 a.a.) protein, derived from the bioluminescent jellyfish *Aequorea victoria*, in which light is produced when energy is transferred from the Ca²⁺-activated photoprotein aequorin to GFP. GFP is acknowledged as a unique tool to monitor dynamic processes in a variety of living cells or organisms. When expressed in either eukaryotic or prokaryotic cells and illuminated by blue or UV light, GFP yields a bright green fluorescence. Light-stimulated GFP fluorescence is species-independent and a fluorescence has been reported from many different types of GFP-expressing hosts, including microbes, invertebrates, vertebrates and plants. Exogenous substrates and cofactors are not required for the fluorescence of GFP, since GFP autocatalytically forms a fluorescent pigment from natural amino acids present in the nascent protein. Additionally, detection of GFP and its variants can be performed with living tissues instead of fixed samples. GFP signals can be quantified by flow cytometry, confocal scanning laser microscopy, and fluorometric assays. Indeed, many recombinant proteins have been engineered with GFP tags to facilitate the detection, isolation and purification of the proteins. The potential applications have been multiplied by the introduction of brighter GFP mutants and mutants with modified spectral properties, like the blue fluorescent protein (BFP), which allow the independent detection of BFP- and GFP- tagged proteins, even when coexpressed in the same cell. Monoclonal antibody reacting specifically with GFP may be useful in various immunotechniques, to identify the expression of a GFP fusion protein *in situ* and by immunoblotting, in bacteria, bacterial lysates or cells and tissues transfected with a GFP fusion protein expressing vectors. It may also be used to correlate levels of GFP protein expression with fluorescence intensity and for immunoprecipitation of GFP fusion proteins.

Source of Antigen and Antibodies

Antigen	Full length GFP form jellyfish aequorea Victoria was expressed and purified (>95%).
Ab Host/type	Balb/c mice were injected with purified GFP and resulting clones were selected to react with GFP. The resulting clone (IgG2a) was expanded as ascites. IgG was purified using ion exchange and protein A/G chromatography
2-Ab	Goat Anti-mouse IgG-HRP conjugate Cat # SA-40320 (AP, biotin, FITC conjugates also available)
-ve control IgG	Cat # SA-20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as -ve control

Full length GFP form jellyfish aequorea Victoria was expressed and purified (>95%). For WB +ve control, **Cat # GFP11-C**, is formulated in SDS-PAGE sample buffer (reduced). This

preparation is not biologically inactive. It is not suitable for ELISA or other applications where native protein is required. It is supplied in 100 ul/vial. For WB, heat once and load 10 ul/lane and visualize with appropriate antibodies (#GFP11-M). If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the GFP11-C solution prior to heating and loading on gels. This preparation is intended for qualitative purpose and not to serve as standard of known concentration. Store frozen in suitable aliquots. Do not freeze, thaw, or heat repeatedly.

Form & Storage

Affinity pure IgG

100 ug/100ul solution lyophilized powder
 Buffer: PBS, pH 7.5, 0.05% sodium azide
Reconstitute powder 100 ul PBS

Storage

Short-term: unopened, undiluted vials for less than a week at 4oC.

Long-term: at -20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Western Blotting (1-2 ug/ml using Chemiluminescence technique). Antibodies react with native and denatured GFP or GFP containing proteins. Full length GFP is ~27-30 kda.

ELISA (0.1-1 ug/ml using 50-100 ng control antigen/well).

Antibody concentration must be optimized for each application under defined experimental conditions.

Specificity and crossreactivity

Monoclonal Anti-GFP recognizes wild type, recombinant, and enhanced form of GFP. Denatured-reduced forms of GFP-fusion proteins in immunoblotting, dot blot and ELISA.

General References: Narayanan, S.R., J. Chromatogr., 658, 237 (1994); Olins, P.O., and Lee, S.C., Curr. Opin. Biotechnol., 4, 520 (1993); Uhlen, M., and Moks, T., Meth. Enzymol., 185, 129, (1990). Tsien, R.Y., Ann. Rev. Biochem., 67, 509 (1998); Chalifie, M., et al., Science, 263, 802 (1994)

*This product is for In vitro research use only.

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