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Product Information

Kit-2 of GFP-tagged Ab

Cat. Number **ABK-GH001-02**

contains: Peroxidase-Mouse Anti-GFP monoclonal Ab (Cat. GH0011) and Rabbit anti-GFP Polyclonal Ab (Cat. Gp0001)

Product Description

Mouse monoclonal Anti-GFP antibody (Cat. Gm0001) is a purified immunoglobulin, IgG1, monoclonal antibody isolated from mouse ascites fluid. Rabbit polyclonal anti-GFP Ab (Cat. Gp0001) is purified from rabbit antiserum. Both Anti-GFP Ab react specifically with GFP tagged recombinant fusion proteins expressed in transfected mammalian cells, from *E. coli* or from *in vitro* translation. Peroxidase-conjugated Mouse anti-GFP monoclonal antibody is useful in ELISA, Immunoblotting and immunoprecipitation. Rabbit anti-GFP polyclonal antibody is also useful in ELISA, Immunoblotting, Immunofluorescent staining and Immunoprecipitation.

Reagent

Supplied in Freeze-dried powder. Resuspended in 0.4 ml 50% glycerol.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store undiluted antibody at -20 °C. Repeated freezing and thawing is not recommended.

Preparation Instructions

Dilute the antibody to 0.2 to 1 μ g/ml in 0.05 M Tris buffered saline (TBS), pH 7.4 or PBS. Adjust the antibody concentration to maximize detection sensitivity.

For monoclonal anti-GFP Ab

Clonality	monoclonal
Clone number	3D8A1B8
Isotype	IgG ₁
Light chain type	kappa
Purity	Protein G purified

Procedures

● Procedure for Western Blot

1. Transfer the GFP fusion protein of interest from *E. coli* or crude mammalian cell lysate of interest to a PVDF membrane.
2. Block the membrane by using a solution of 5% non-fat dry milk in TBS or PBS at room temperature for 5 minutes to 1 hour.
3. Wash the membrane three for 5 minutes each in TBS or PBS with 0.05% Tween 20 (Wash buffer-WB) at room temperature.
4. Incubate the membrane with Peroxidase-conjugated Mouse anti- GFP monoclonal antibody as the primary

antibody at 0.2 to 1 μ g/ml with 5% non-fat dry milk of Wash Buffer-WB at room temperature for 60 minutes (do not exceed 2 hours). Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

5. Wash the membrane three times for 5 minutes each in Wash buffer-WB at room temperature with agitation.
 6. Treat the membrane with chemiluminescent, or other peroxidase substrate.
- *Procedure for Immunofluorescent staining*
 1. Culture mammalian cells on cover slip for proper time. Fix the cover slip with cold acetone/ methanol (1:1) for 90 seconds or 4% paraformaldehyde for 15 minutes.
 2. Dilute the antibody to 0.4 to 1 μ g/ml in PBS with 0.05% Tween 20 (Wash buffer-IF). After soaking in PBS with 2.5% BSA, cells were stained with Rabbit Anti-GFP Polyclonal Antibody at 37 °C for 30 minutes to overnight.
 3. Wash the cell by incubating in Wash buffer-IF at room temperature for three times, each 5 minutes.
 4. Incubate the cells with FITC Anti-Rabbit IgG as the secondary antibody in Wash buffer-IF at room temperature for 30 minutes to 1 hour.
 5. Wash the cover slip for three times as previous. Mount the cells on 50% to 90% glycerol and visualize the cells on fluorescent microscope.
 - *Procedure for Immuno-precipitation*
 1. Incubate 0.1 to 1 mg crude cell extract with 2 to 20 μ g Rabbit Anti- GFP polyclonal Ab (Cat. Gp0001) in 0.5 ml buffer with proper protease inhibitors at 4 °C for 1 hour with agitation.
 2. Add 15 μ l of 50% slurry of Protein A Sepharose bead and incubate for 30 minutes with agitation.
 3. Centrifuge the vial 7000 rpm for 1 minute, and carefully aspirate the supernatant without disturbing the beads.
 4. Add 0.5 to 1 ml PBS with 0.1 % Triton (Wash buffer-IP), after inverting for several times or for 3 min, centrifuge again as Step 3.
 5. Repeat Step 3 & 4 for four to six times.
 6. Add sample dye and blot onto PVDF membrane as western blot analysis.
 7. After blocking in 5% non-fat dry milk in Wash buffer-IP, use proper primary antibody such as Peroxidase-conjugated Mouse anti-GFP monoclonal antibody (Cat. GH0001) in Wash buffer-IP and incubate for 45 minutes.
 8. Treat the membrane with chemiluminescent, or other peroxidase substrate.