#20014

Anti-GFP-Tag mAb (Agarose conjugated)

☐ 500 µl (10-25 immunoprecipitations)
☐ 1000 µl (20-50 immunoprecipitations)
☐ 5 ml (100-250 immunoprecipitations)

Orders ■ 400-6123-828
orders@ab-mart.com
Web ■ www.ab-mart.com.cn

BACKGROUND

The green fluorescent protein (GFP) was originally identified as a protein involved in the bioluminescence of the jellyfish Aequorea victoria. GFP cDNA produces a fluorescent product when expressed in prokaryotic cells, without the need for exogenous substrates or cofactors, making GFP a useful tool for monitoring gene expression and protein localization in vivo. Several GFP mutants have been developed, including EGFP, which fluoresce more intensely than the wildtype GFP and have shifted excitation maxima, making them useful for FACS and fluorescence microscopy as well as double-labeling applications. GFP is widely used in expression vectors as a fusion protein tag, allowing expression and monitoring of heterologous proteins fused to GFP.

REFERENCES

1. Prasher, D.C., et al. 1992. Primary structure of the Aequorea victoria green fluorescent protein. Gene 111: 229-233.
2. Chalfie, M., et al. 1994. Green fluorescent protein as a marker for gene expression. Science 263: 802-805.

SOURCE

This Abmart monoclonal antibody is produced by immunizing animals with full-length recombinant GFP.

SPECIFICITY

GFP-Tag (7G9) Mouse mAb detects GFP and GFP fusion proteins.

STORAGE

The product is supplied as a 50% slurry in storage buffer (1 PBS, pH 7.4, containing 0.1% NaN). Store the product at 4°C and do not freeze.

REACTIVITY

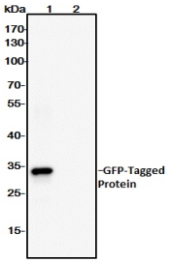
All

ISOTYPE

Mouse IgG1

RECOMMENDED ELUTION BUFFER

0.2 M Glycine, pH 2.2



HEK 293T cells were transfected with GFP-tagged protein or not, and 100 µl cell lysate (about 100 µg total protein) was incubated with 30 µl 50% slurry of Anti-GFP Agarose for 3 h at 4°C. After washing, the beads were eluted by 30 µl elution buffer twice. After neutralization of the eluant, 6 µl 6× SDS loading buffer was added. Then 20 µl sample was subjected to the SDS-PAGE. Blot was probed with Anti-GFP-Tag Mouse mAb.
Lane 1: 1st Elution with elution buffer.
Lane 2: IP of untransfected HEK 293T lysate.

IMMUNOPRECIPITATION PROCEDURE

The work can be performed in 1.5 ml micro-centrifuge tubes or in spin columns.

1. Thoroughly resuspend the Anti-GFP Agarose by inverting the tube or vial several times.
2. Add 20-50 µl 50% slurry of Anti-GFP Agarose into cell lysate using a wide-bore pipette tip.
   Note: The lysate should be fresh, and for a well expressed tagged protein, 200 µl lysate (200-500 µg total protein) usually yields a good IP result.
3. Incubate with gentle mixing for 2 h to overnight at 4°C.
4. Wash the beads with 1 ml TBS buffer or lysis buffer, such as RIPA (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate), centrifuge for 3 min at 2,000× g, and discard the supernatant. Wash 3 times, avoid losing beads during washes.
5. Elution of the GFP tagged protein.
   Option 1. Elution with elution buffer.
   Add 30-50 µl elution buffer to the beads, gently tap the tube to mix well, immediately centrifuge for 3 min, transfer the supernatant very carefully to a fresh tube (Avoid transferring any beads).
   Note: Neutralize the eluant immediately by add 1 µl of 1.5 M Tris, pH 9.0 per 20 µl Elution buffer.
   Option 2. Elution with GFP peptide
   Add 30-50 µl GFP peptide solution (100 µg/ml GFP peptide in TBS buffer), gently tap the tube to mix well, incubate for 10 min, centrifuge for 3 min, and transfer the supernant to a fresh tube. TBS buffer: 50 mM Tris HCl, 150 mM NaCl, pH 7.4.
   Option 3. Elution with SDS loading buffer
   Add 30 µl 2× SDS loading buffer, gently tap the tube to mix well, boil at 100°C for 5 min, centrifuge for 3 min, transfer the supernatant to a fresh tube.
   Note: in this case, the supernatant contains not only the binding proteins, but also IgG (heavy and light chains).
6. Prepare SDS-PAGE gel for western blotting or proceed to other assays.

COMPANION PRODUCTS

#M20001 His-Tag (2A8) mAb
#M20002 Myc-Tag (19C2) mAb
#M20003 HA-Tag (26D11) mAb
#M20004 GFP-Tag (7G9) mAb
#M20007 GST-Tag (12G8) mAb
#M20008 DYKDDDDK-Tag (3B9) mAb
(Same as Sigma's Anti-FLAG?)
#M20053 HA-Tag (3L17) mAb
#M30111 His-Tag (10E2) mAb
#M20012 Anti-Myc-Tag mAb (Agarose conjugated)
#M20013 Anti-HA-Tag mAb (Agarose conjugated)
#M20018 Anti-DYKDDDDK-Tag mAb (Agarose conjugated)
(Same as Sigma's Anti-FLAG?)
#M20153 Anti-HA-Tag mAb (Agarose conjugated)

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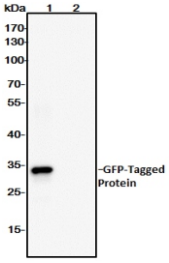

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For *in vitro* research use only and not intended for use in humans or animals.