

Abking Biotechnologies Inc.
Add: Rm. 2, 13F., No.25, Ln. 169, Kangning St., Xizhi Dist.,
New Taipei City 221, Taiwan
Tel: 886-2- 2692-4275 Fax: 886-2- 2692-4539
E-mail: abking@abk.com.tw

Product Information

Rabbit Anti-HA Polyclonal Ab

Produced in rabbit, purified immunoglobulin

Catalog Number **Hp0001-02 (0.2mg/vial)**

Product Description

Rabbit polyclonal Anti-HA antibody is a purified immunoglobulin isolated from rabbit antiserum.

Anti-HA reacts specifically with HA tagged recombinant fusion proteins expressed in transfected mammalian cells, from *E. coli*, or from in vitro translation. Rabbit anti-HA 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 which contains 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.05% sodium azide and 2 mg/ml BSA.

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 2.5 $\mu\text{g/ml}$ in 0.05 M Tris buffered saline (TBS), pH 7.4 or PBS. Adjust the antibody concentration to maximize detection sensitivity.

Product Profile

Antigenic binding site: N-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-C.

Clonality polyclonal

Purity Protein A purified

Procedures

● Procedure for Western Blot

1. Transfer the HA fusion protein of interest from *E. coli* or crude mammalian cell lysate 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 times for 5 minutes each in TBS or PBS with 0.05% Tween 20 (Wash Buffer-WB) at room temperature.
4. Incubate the membrane with rabbit anti- HA Ab (Hp0001) as the primary antibody at 0.2 to 1 $\mu\text{g/ml}$ in with 5% non-fat dry milk of Wash Buffer-WB at room temperature for 60 minutes.
5. Wash the membrane three times for 5 minutes each in Wash Buffer-WB at room temperature with agitation.
6. Incubate the membrane with Anti-rabbit IgG Peroxidase as the secondary antibody at the manufacturer's recommended concentration in with 5% non-fat dry milk of Wash Buffer-WB. Incubate at room temperature for 40 minutes (do not exceed 2 hours). Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
7. Wash the membrane three times for 15 minutes each in Wash buffer-WB at room temperature.
8. 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 $\mu\text{g/ml}$ in PBS with 0.05% Tween 20 (Wash Buffer-IF). After washing in PBS with 0.5% BSA, cells

were stained with anti- HA Ab 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 steps. 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 5 μg rabbit Anti- HA polyclonal Ab in 0.5 ml buffer PBS with 0.1 % to 0.5% Triton (Wash buffer-IP) with proper protease inhibitors at 4°C for 1 hour with agitation.
 2. Add 15 μl of 50% slurry of Protein A (or Protein G) 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 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 TBS or PBS, use proper primary antibody such as Anti- HA monoclonal Ab or anti-target from mouse or other species in with 5% non-fat dry milk of Wash Buffer-WB. and incubate for 45 minutes to overnight.
 8. After wash for three times with agitation, incubate the membrane with secondary Ig such as Goat Anti-mouse Ig Peroxidase in Wash buffer-IP and incubate for 40 minutes (do not exceed 2 hours).
 9. Treat the membrane with chemiluminescent, or other peroxidase substrate.