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

Mouse Anti-HA Monoclonal Ab Clone 2F1-1H7

produced in mouse, purified immunoglobulin
Catalog Number **Hm0001-02 (0.2mg/vial)**

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Product Description
Mouse Anti-HA Monoclonal Antibody is a purified immunoglobulin, IgG2b, monoclonal antibody isolated from mouse ascites fluid. Anti-HA reacts specifically with HA tagged recombinant fusion proteins expressed in transfected mammalian cells, from E. coli or from in vitro translation. Mouse anti-HA monoclonal 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 ℃. Repeated freezing and thawing is not recommended.

Preparation Instructions
Dilute the antibody to 0.2 to 2.5 μg/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.

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Clonality monoclonal
Clone number 2F1-1H7
Isotype IgG_{2b}
Light chain type kappa
Purity Protein G purified

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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 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 Monoclonal anti-HA Ab (Hm0001) as the primary antibody at 0.2 to 1 μg/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-mouse 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 μg/ml in PBS with 0.05% Tween 20 (Wash Buffer-IF). After soaking in PBS with 2.5% BSA, cells were stained with anti- HA Ab at 37 ℃ 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- mouse 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 mouse Anti-HA monoclonal Ab in 0.5 ml buffer PBS with 0.1 % to 0.5% Triton (Wash buffer-IP) with proper protease inhibitors at 4℃ for 1 hour with agitation.

2. Add 15 μl of 50% slurry of 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 minutes, 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 polyclonal 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-rabbit 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