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Product Information

Kit-2 of HA-tagged Ab

Cat. Number **ABK-HH001-02**
contains: Peroxidase-Mouse Anti-HA monoclonal Ab (Cat. HH0011) and Rabbit anti-HA Polyclonal Ab (Cat. Hp0001)

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Product Description
Mouse monoclonal Anti-HA antibody (Cat. Hm0001) is a purified immunoglobulin, IgG2b, monoclonal antibody isolated from mouse ascites fluid. Rabbit polyclonal anti-HA Ab (Cat.Hp0001) is purified from rabbit antiserum.
Both Anti-HA Ab react specifically with HA tagged recombinant fusion proteins expressed in transfected mammalian cells, from E. coli or from in vitro translation. Peroxidase-conjugated Mouse anti-HA monoclonal antibody is useful in ELISA, Immunoblotting and immunoprecipitation. 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.

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 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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For monoclonal anti-HA Ab

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 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- HA monoclonal antibody as the primary antibody at 0.2 to 1μg/ml with 5% non-fat dry milk of Wash Buffer-WB at room temperature for 60 minutes (do not exceed 2 hr). 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 μg/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- HA 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 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 20 μg Rabbit Anti- HA polyclonal Ab (Cat. Hp0001) in 0.5 ml buffer with proper protease inhibitors at 4 °C for 1 hour with agitation.
2. Add 15 μl 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 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 Wash buffer-IP, use proper primary antibody such as Peroxidase-conjugated Mouse anti-HA monoclonal antibody (Cat. HH0001) in Wash buffer-IP and incubate for 45 minutes.
8. Treat the membrane with chemiluminescent, or other peroxidase substrate.