Abking Biotechnologies Inc.
Add: 5F-2, 669, Sec 5, Chunghsiao E. Road, Taipei 110, Taiwan.
Tel: 886-2- 8785-8136
Fax: 886-2- 8785-8103
E-mail: abking@abk.com.tw

Product Information

Kit-2 of FLAXNC-tagged Ab

Cat. Number **ABK-FH001-02**
contains: Peroxidase-Mouse Anti-FLAXNC monoclonal Ab (Cat. FH0011) and Rabbit anti-FLAXNC Polyclonal Ab (Cat. Fp0001)

****

Product Description
Mouse AntiI-FLAXNC (FLAG) monoclonal antibody (Cat. Fm0001) is a purified immunoglobulin, IgG1, monoclonal antibody isolated from mouse ascites fluid. Rabbit anti-FLAXNC polyclonal Ab (Cat.Fp0001) is purified from rabbit antiserum. Both Ab react specifically with FLAG tagged recombinant fusion proteins expressed in transfected mammalian cells, from E. coli or from in vitro translation. Peroxidase-conjugated Mouse anti-FLAXNC monoclonal antibody is useful in ELISA, Immunoblotting. Rabbit anti-FLAXNC 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-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C

****

For monoclonal anti-FLAXNC Ab

Clonality	monoclonal
Clone number	2F11-4H10
Isotype	IgG_1
Light chain type	kappa
Purity	Protein G purified

****

Procedures

- *Procedure for Western Blot*

1. Transfer the FLAG 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 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 Peroxidase-conjugated Mouse anti-FLAXNC monoclonal antibody (Cat. FH0011) 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 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 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-FLAG 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 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-FLAXNC polyclonal Ab (Cat. Fp0001) 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- FLAXNC monoclonal antibody (Cat. FH0001) in Wash buffer-IP and incubate for 45 minutes.
8. Treat the membrane with chemiluminescent, or other peroxidase substrate.