For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-Selenium Binding Protein

Code No.	Clone	Subclass	Quantity	Form
M061-3	4D4	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: Selenium is an essential trace element that has been recognized to have a capacity for conferring tolerance to the toxic manifestation of various heavy metal exposures (for example, cadmium, mercury, lead and arsenic etc.) and chemical substances including radicals. Dietary selenium supplementation within the range of normal dietary intake can significantly decrease the incidence and mortality of several cancers. Moreover, it is reported deficiency of it may cause certain neurological diseases including schizophrenia. These effects of selenium are thought to be emerged by the proteins that accept the selenium and the presence of such proteins has been documented in numerous organs. These proteins are comprised of selenoproteins that accept the selenium as selenocystein and proteins that bind selenium ion(s) after synthesis. Mouse 56 kDa selenium binding protein (mouse SBP, mSP56) is found as one of such proteins that bind selenium after its synthesis. It is a cytosolic protein whose physiological property is largely unknown. Mouse SBP is extremely homologous to another 56 kDa mouse protein named acetaminophen binding protein (mouse AcBP) which binds the metabolite of acetaminophen concerning serious liver injury, suggesting that both 56 kDa proteins may be involved in detoxification processes. Human 56 kDa selenium binding protein (human SBP) is a protein that has higher homology to 56 kDa mouse SBP rather than to mouse AcBP. Human SBP is expressed in liver, lung and kidney at high level and in heart and intestine at lower level.

SOURCE: This antibody was purified from hybridoma (clone 4D4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with the recombinant full-length human SBP.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with Selenium Binding Protein (SBP) on Western blotting.

INTENDED USE:
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APPLICATIONS:
Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; 2 µg/mL
Immunocytochemistry; Not tested
Flow cytometry; Not tested

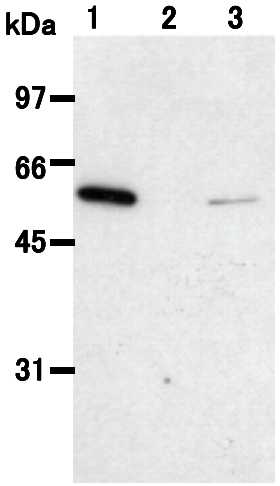
Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	ZR75-1, KB	WR19L, Ba/F3	COS7
Reactivity on WB	+	+ weak	+

REFERENCES:
1) Glatt, S. J., *et al.*, *PNAS* **102**, 15533-15538 (2005)
2) Chang, P. M., *et al.*, *J.Cell Biochem.* **64**, 217-224 (1997)
3) Lanfear, J., *et al.*, *Carciogenesis* **14,** 335-341 (1993)
4) Pumford, N. R., *et al.*, *Biochem. Biophys Res. Commun.* **182**, 1348-1355 (1992)
5) Bansal, M. P., *et al.*, *Carcinogenesis* **11**, 2071-2073 (1990)
6) Bansal, M. P., *et al.*, *Carcinogenesis* **10**, 541-546 (1989)

Clone 4D4 is used in reference number 1).



Western blot analysis of Bad expression in ZR75-1 (1), HL-60 (2) and HeLa (3) using M061-3 at 1 µg/mL.

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PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; ZR75-1)

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 μL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; human primary lung adenocarcinoma)