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Not for use in diagnostic procedures.

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Anti-HA-tag mAb-HRP-DirecT

CODE No.	M180-7
CLONALITY	Monoclonal
CLONE	TANA2
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 μL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, YPYDVPDYA (HA-tag)
REACTIVITY	This antibody reacts with N-terminal and C-terminal HA-tagged proteins.
FORMULATION	PBS/Preservative/Stabilizer
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATION

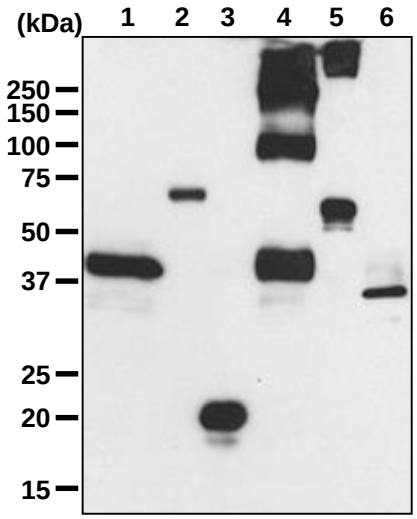
Western blotting	1:5,000-1:10,000

For more information, please visit our web site **https://ruo.mbl.co.jp/**.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

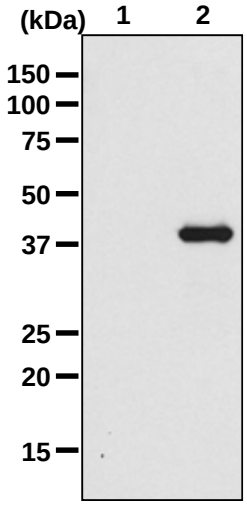
1) Wash 1 x 10^6 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3).
8) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
9) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



Western blotting analysis of HA-tagged protein

Lane 1: Met-N-terminal HA-tagged protein A
Lane 2: Met-N-terminal HA-tagged protein B
Lane 3: Met-N-terminal HA-tagged protein C
Lane 4: N-terminal HA-tagged protein D
Lane 5: N-terminal HA-tagged protein E
Lane 6: C-terminal HA-tagged protein F

Immunoblotted with M180-7



Western blottting analysis for immunoprecipitated sample from transfectant

Lane 1: IP with Mouse IgG2b (isotype control) (MBL, code no. M077-3)
Lane 2: IP with Anti-HA-tag mAb (MBL, code no. M180-3)

Immunoblotted with M180-7