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

Anti-HA-tag HRP-DirecT

Code No.	Quantity	Antibody type
561-7	100 μL	Rabbit Polyclonal

HRP-DirecT Series

HRP-DirecT is a series of HRP conjugated primary antibodies developed by MBL.

HRP-DirecT Series products don't need secondary antibodies. That brings the following advantages:

① Total incubation time is cut in half.

The reaction with the secondary antibody becomes unnecessary. In the shaking method, reaction time is only 30 minutes. Spend your evening doing more important things.

② Clear result

No more heavy and light chain bands in Immunoprecipitation. The HRP conjugated primary antibody will not detect your precipitating antibody. Clear result helps your research.

Usually, the secondary antibody amplifies the signal of an unconjugated primary antibody. Therefore, it is thought to be difficult to obtain a strong signal with a directly conjugated antibody. To overcome this hurdle, MBL has improved the HRP conjugation method. MBL's HRP-DirecT Series yield strong signal with minimum background. Give it a try - the HRP-DirecT Series will not disappoint you.

DETECTED ANTIGEN

HA-tag

REAGENT

PBS/Preservative/Stabilizer

STORAGE

This antibody is stable for one year from the date of purchase when stored at 4°C.

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APPLICATION

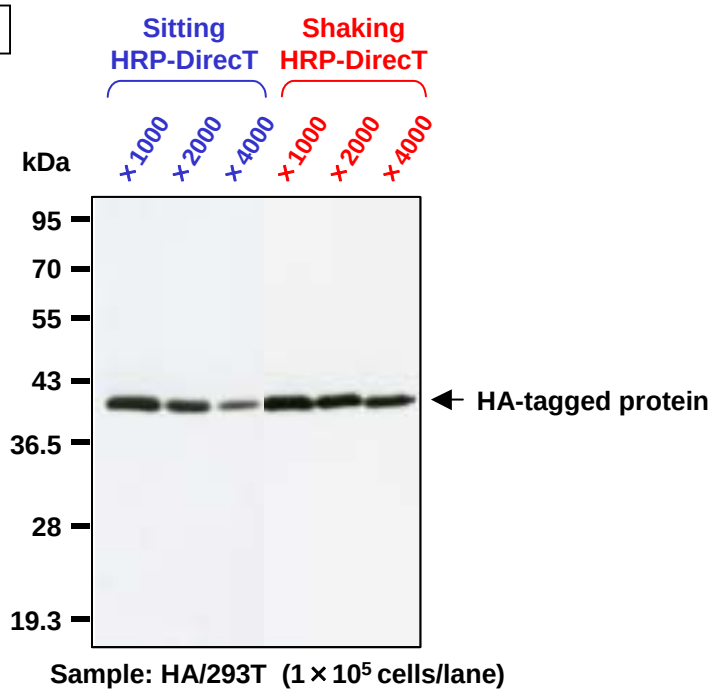
Western blotting

Method	Reaction times	Dilution	Detaction
Sitting	1hour	1,000～4,000	chemiluminescence
Shaking	30minutes	1,000～4,000	chemiluminescence

＊ Detailed procedure is provided in the following protocols.

Comparison of Sitting method and Shaking method

Figure1



Immunocytochemistry; Not tested

Immunohistochemistry; Not tested

Immunoprecipitation（for clear results）

There are two causes of non-specific bands at Western blotting that follows Immunoprecipitation.

Cause1: Reaction of the secondary antibody with the antibody used at IP. (Fig.2; lane 1)

Solution

MBL HRP-DirecT series (HRP conjugated primary antibodies products series) does not detect the precipitating antibody (Fig. 2; lanes 2*, 3, and 4). *The nonspecific band in lane 2 represents protein A/G. Please refer to **Cause 2**.

Cause 2: Reaction of protein A/G with the primary and secondary antibodies used at WB. (Fig.2; lane 2)

Boiling the Immunoprecipitated sample with Laemmli's sample buffer; this causes dissociation of protein A/G from its carrier. Therefore, the primary and secondary antibodies bind to protein A/G at western blotting.

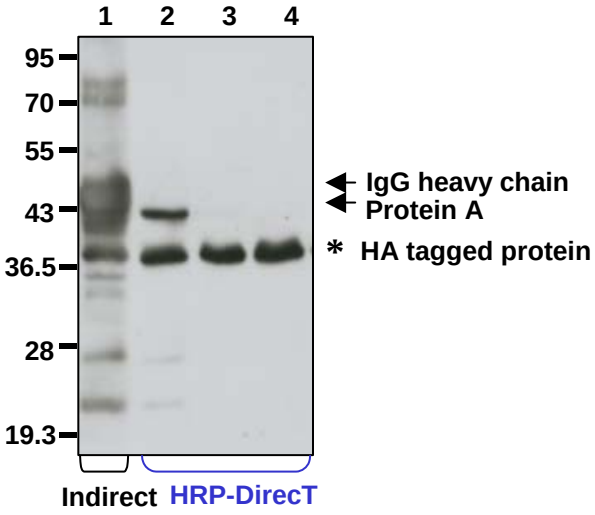
Solution

①Add Clear Back for IP Western (MBL; code no. MTG-002) in 1:100 ratio to the diluted HRP-DirecT. (Fig.2; lane 3)

②Use an Agarose-conjugated antibody for Immunoprecipitation. (Fig.2; lane 4)

Figure2

IP Antibody
1: anti-HA-tag (code no. 561) + Protein A
2: anti-HA-tag (code no. 561) + Protein A
3: anti-HA-tag (code no. 561) + Protein A + Clear Back for IP Western
4: anti-HA-tag-Agarose (code no. 561-8)



PROTOCOL

Western Blotting

1. SDS-PAGE and Western Blotting

   Perform SDS-PAGE electrophoresis on the protein samples and transfer the protein to a PVDF membrane according to standard techniques.

2. Blocking

   Block the membrane with 5% skimmed milk in PBS (or TBS). Incubate for 1 hour with shaking at room temperature or over night at 4°C.

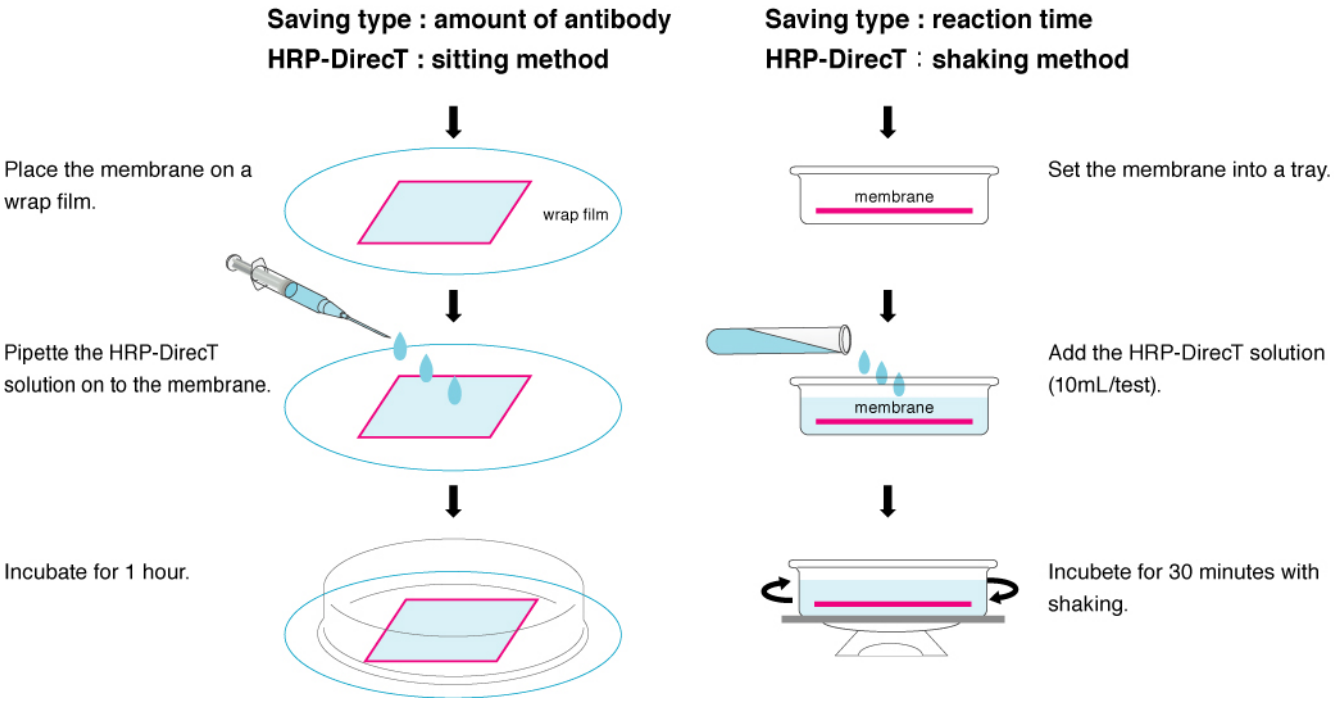
3. Incubate the membrane with HRP-DirecT antibody as follows:

① Sitting Method (Saving type: amount of antibody)

Dilute the HRP-DirecT antibody with 1% skimmed milk in PBS (or TBS). Use at least 30 µL/ cm^2 membrane. Antibody dilution ratio is suggested in the APPLICATION. Drain the excess blocking buffer from the membranes and place them, protein side up, on a wrap film or other suitable clean surface. Pipette the HRP-DirecT solution on to the membrane. Cover the membrane with plastic case to avoid drying. Incubate for 1 hour at room temperature.

② Shaking Method (Saving type: reaction time)

Dilute the HRP-DirecT antibody with 1% skimmed milk in PBS (or TBS). Use at least 10mL. Antibody dilution ratio is suggested in the APPLICATION. Transfer membrane to a tray containing HRP-DirecT antibody. Incubate for 30 minutes with shaking at room temperature.



4. Washing

Wash the membrane in PBS (or TBS） with 0.05% Tween-20 three times for 5 minutes each.

5. Detection

Detect with a chemiluminescence substrate according to the manufacturer’s instructions.