

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

**RiboCluster Profiler™**

RBP Antibody

# Anti-AHCY (SAHH) pAb

<b>CODE No.</b>	RN126PW
<b>CLONALITY</b>	Polyclonal
<b>ISOTYPE</b>	Rabbit Ig, affinity purified
<b>QUANTITY</b>	100 µL
<b>SOURCE</b>	Purified Ig from rabbit serum
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.
<b>APPLICATIONS</b>	
<u>Western blotting</u>	1:1,000
<u>Immunoprecipitation</u>	5 µL/500 µL of cell extract from 5 x 10 <sup>6</sup> cells/sample

## SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster	Thale Cress*
Cells	HeLa, HEK293T, Jurkat, K562	NIH/3T3, WR19L	Rat1	CHO	
Reactivity	+	+	+	+	

\*Information from the customer. (Not tested by MBL)

**Entrez Gene ID** 191 (Human), 269378 (Mouse), 29443 (Rat), 100750969 (Hamster)

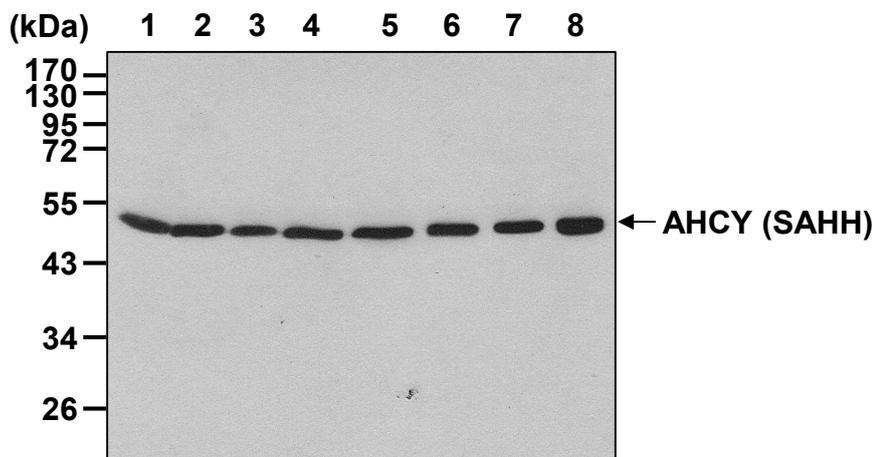
For more information, please visit our website at <https://ruo.mbl.co.jp/>.

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

### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
- 2) Boil the samples for 3 min. and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 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 (10 min. x 3 times).
- 8) Incubate the membrane with the 1:5,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 9) Wash the membrane with PBS-T (10 min. x 3 times).
- 10) Wipe excess buffer on the membrane, 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.
- 11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual settings. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, HEK293T, Jurkat, K562, NIH/3T3, WR19L, Rat1 and CHO)



#### ***Western blot analysis of AHCY (SAHH)***

- Lane 1: HeLa
- Lane 2: HEK293T
- Lane 3: Jurkat
- Lane 4: K562
- Lane 5: NIH/3T3
- Lane 6: WR19L
- Lane 7: Rat1
- Lane 8: CHO

Immunoblotted with Anti-AHCY (SAHH) pAb (RN126PW)

## **Immunoprecipitation**

- 1) Wash  $1 \times 10^7$  cells 4 times with PBS and resuspend them with 1 mL of ice-cold Lysis Buffer (+) (MBL; code no. RN1001) containing appropriate protease inhibitors and DTT. Vortex thoroughly, then incubate on ice for 10 min.
- 2) Centrifuge the tube at  $12,000 \times g$  for 5 min. at  $4^\circ\text{C}$  and transfer the supernatant to another tube.
- 3) Add 20  $\mu\text{L}$  of 50% protein G agarose beads slurry resuspended in ice-cold Wash Buffer (+) (MBL; code no. RN1001) containing DTT at the appropriate concentration into the supernatant. Incubate it at  $4^\circ\text{C}$  with rotating for 1 hr.
- 4) Centrifuge the tube at  $2,000 \times g$  for 1 min. at  $4^\circ\text{C}$  and transfer the supernatant to another tube (precleared sample).
- 5) Mix 20  $\mu\text{L}$  of 50% protein G agarose beads slurry resuspended in 1 mL of ice-cold Wash Buffer (+) with Normal Rabbit IgG (RIP-Assay Kit) or Anti-AHCY (SAHH) pAb (MBL; code no. RN126PW) as suggested in the **APPLICATIONS**. Incubate at  $4^\circ\text{C}$  with rotating for 1 hr.
- 6) Wash the beads 1 time with ice-cold Lysis Buffer (+). Carefully discard the supernatant.
- 7) Add 500  $\mu\text{L}$  of the precleared sample (prepared in step 4)) to the tube containing antibody conjugated beads, then incubate with gentle agitation for 2 hr. at  $4^\circ\text{C}$ .
- 8) Wash the bead pellet 4 times with 1 mL of ice-cold Wash Buffer (+).
- 9) Resuspend the bead pellet in 20  $\mu\text{L}$  of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 10) Load 20  $\mu\text{L}$  of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 11) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at  $1 \text{ mA}/\text{cm}^2$  for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 12) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) overnight at  $4^\circ\text{C}$ .
- 13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min.  $\times$  3 times).
- 14) 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.)
- 15) Wash the membrane with PBS-T (10 min.  $\times$  3 times).
- 16) Incubate the membrane with 1:1,000 of Rabbit TrueBlot<sup>®</sup> anti-Rabbit IgG-HRP (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 17) Wash the membrane with PBS-T (10 min.  $\times$  3 times).
- 18) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min.
- 19) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 20) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; HeLa)

