

**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, 1 mg/mL
<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 for chemiluminescence detection system
<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 web site <http://ruo.mbl.co.jp/je/rip-assay/>



MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

URL <http://ruo.mbl.co.jp/je/rip-assay/>

e-mail [support@mbi.co.jp](mailto:support@mbi.co.jp), TEL 052-238-1904

## RELATED PRODUCTS

### RIP-Assay Kit

RN1001	RIP-Assay Kit
RN1005	RIP-Assay Kit for <i>microRNA</i>

### RIP-Certified Antibody

RN001P	Anti-EIF4E pAb
RN002P	Anti-EIF4G1 (Human) pAb
RN003P	Anti-EIF4G2 pAb
RN004P	Anti-ELAVL1 (HuR) pAb
RN005P	Anti-ELAVL2 (HuB) (Human) pAb
RN006P	Anti-ELAVL3 (HuC) pAb
RN007P	Anti-IGF2BP1 (IMP1) pAb
RN008P	Anti-IGF2BP2 (IMP2) pAb
RN009P	Anti-IGF2BP3 (IMP3) pAb
RN010P	Anti-MSI1 (Musashi1) pAb
RN011P	Anti-PTBP1 (Human) pAb
RN012P	Anti-STAU1 (Human) pAb
RN013P	Anti-STAU2 (Human) pAb
RN015P	Anti-YBX1 pAb
RN019P	Anti-HNRNPK pAb
RN020P	Anti-ILF3 (Human) pAb
RN021P	Anti-KHDRBS1 pAb
RN022P	Anti-PABPC4 pAb
RN024P	Anti-PCBP1 pAb
RN025P	Anti-PCBP2 pAb
RN026P	Anti-PUM1 pAb
RN027P	Anti-PUM2 pAb
RN028P	Anti-EIF2C1 (AGO1) pAb
RN032P	Anti-CIRBP pAb
RN033P	Anti-TNRC6A (GW182) (Human) pAb
RN037P	Anti-AUH pAb
RN038P	Anti-CPEB1 pAb
RN041P	Anti-KHDRBS2 (SLM1) pAb
RN045P	Anti-SLBP pAb
RN001M	Anti-IGF2BP1 (IMP1) mAb (6H6)
RN003M	Anti-EIF2C2 (AGO2) (Human) mAb (1B1-E2H5)
RN004M	Anti-Ribosomal P0/P1/P2 mAb (9D5)
RN005M	Anti-EIF2C2 (AGO2) mAb (2A8)
RN006M	Anti-EIF4E mAb (C107-3-5)
RN007M	Anti-ELAVL1 (HuR) mAb (C67-1)
RN009M	Anti-PABPC1 mAb (10E10)

### RBP Antibody

RN008MW	Anti-ELAVL1 (HuR) mAb (C54-6)
RN010MW	Anti-PIWIL1 (MIWI) mAb (2D9)
RN023PW	Anti-PABPN1 pAb
RN047PW	Anti-PTBP2 pAb
RN050PW	Anti-GRSF1 pAb
RN051PW	Anti-HDLBP (Vigilin) pAb
RN052PW	Anti-HNRNPC pAb
RN054PW	Anti-PCBP3 pAb
RN060PW	Anti-HNRNPD (AUF1) pAb
RN061PW	Anti-HNRNPA0 pAb
RN063PW	Anti-DHX9 pAb
RN064PW	Anti-FUSIP1 (SRSF10) pAb
RN065PW	Anti-KHSRP pAb
RN067PW	Anti-PPP1R10 pAb
RN068PW	Anti-PPP1R8 pAb
RN069PW	Anti-RBM14 pAb

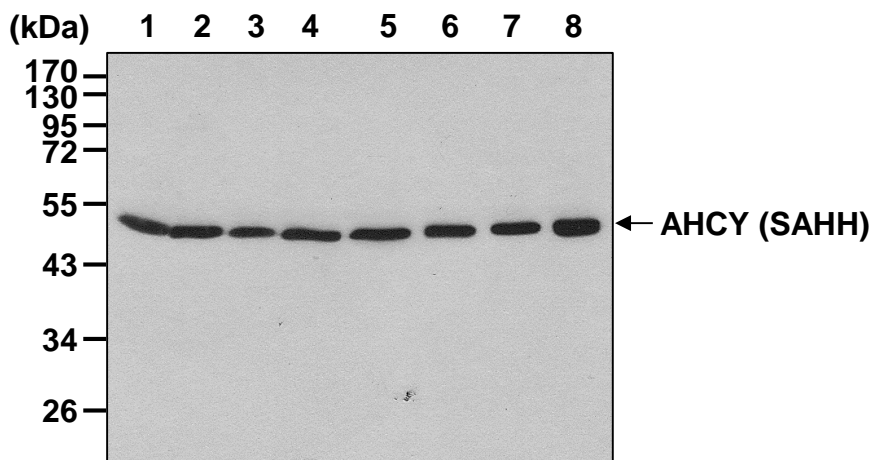
RN077PW	Anti-SMN1 pAb
RN078PW	Anti-SMNDC1 pAb
RN079PW	Anti-SRSF7 (9G8) pAb
RN080PW	Anti-SRSF3 (SRp20) pAb
RN081PW	Anti-SRSF9 (SRp30c) pAb
RN082PW	Anti-SRSF5 (SRP40) pAb
RN084PW	Anti-SRRM1 (SRM160) pAb
RN085PW	Anti-U2AF1 pAb
RN086PW	Anti-U2AF2 pAb
RN087PW	Anti-ALYREF (THOC4) pAb
RN088PW	Anti-NXF1 (TAP) pAb
RN089PW	Anti-MAGOH pAb
RN090PW	Anti-DDX21 pAb
RN091PW	Anti-DDX23 pAb
RN092PW	Anti-NONO (P54NRB) pAb
RN093PW	Anti-PRPF4 pAb
RN094PW	Anti-PRPF8 pAb
RN095PW	Anti-SNRNP200 pAb
RN096PW	Anti-SNRNP40 pAb
RN097PW	Anti-SNRNP70 pAb
RN098PW	Anti-EDC4 pAb
RN099PW	Anti-EIF4A1 pAb
RN100PW	Anti-EXOSC5 (RRP46) (Human) pAb
RN101PW	Anti-FBL (Fibrillarin) pAb
RN102PW	Anti-GEMIN2 (Human) pAb
RN103PW	Anti-NCBP1 (CBP80) pAb
RN104PW	Anti-PAN2 (USP52) (Human) pAb
RN105PW	Anti-PARN pAb
RN106PW	Anti-SFPQ (PSF) pAb
RN107PW	Anti-TARDBP (TDP-43) pAb
RN108PW	Anti-UPF1 pAb
RN109PW	Anti-XRN1 (Human) pAb
RN110PW	Anti-CNOT7 (CAF1) pAb
RN111PW	Anti-ETF1 (eRF1) pAb
RN112PW	Anti-DCP1B (Human) pAb
RN113PW	Anti-DHX36 (RHAU) pAb
RN114PW	Anti-HNRNPA1 pAb
RN115PW	Anti-LIN28B (Human) pAb
RN116PW	Anti-DDX39B (UAP56) pAb
RN117PW	Anti-CCAR2 (DBC1) pAb
RN118PW	Anti-UPF3B pAb
RN119PW	Anti-GSPT2 (eRF3b) (Human) pAb
RN120PW	Anti-RBM8A (Y14) pAb
RN121PW	Anti-FTO (Human) pAb
RN122PW	Anti-ALKBH5 pAb
RN123PW	Anti-YTHDF2 pAb
RN124PW	Anti-RNMT (Human) pAb
RN125PW	Anti-HENMT1 pAb
RN127PW	Anti-NSUN2 (Human) pAb
RN128PW	Anti-TRMT6 (Human) pAb
RN129PW	Anti-DDX6 (RCK/p54) pAb
RN130PW	Anti-TRMT61A (Human) pAb
RN016M	Anti-7-methylguanosine (m <sup>7</sup> G)-Cap mAb (150-15)
RN017M	Anti-7-methylguanosine (m <sup>7</sup> G) mAb (4141-13)
RN019M	Anti-2,2,7-trimethylguanosine (m <sub>3</sub> G/TMG) mAb (235-1)
D345-3	Anti-1-methyladenosine (m <sup>1</sup> A) mAb (AMA-2)
D346-3	Anti-5-methylcytidine (m <sup>5</sup> C) mAb (FMC-9)

For the latest information of RiboCluster Profiler™, please visit our website at <http://ruo.mbl.co.jp/je/rip-assay/>

### **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 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Add 20  $\mu$ 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°C with rotating for 1 hr.
- 4) Centrifuge the tube at 2,000 x g for 1 min. at 4°C and transfer the supernatant to another tube (precleared sample).
- 5) Mix 20  $\mu$ 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°C with rotating for 1 hr.
- 6) Wash the beads 1 time with ice-cold Lysis Buffer (+). Carefully discard the supernatant.
- 7) Add 500  $\mu$ 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°C.
- 8) Wash the bead pellet 4 times with 1 mL of ice-cold Wash Buffer (+).
- 9) Resuspend the bead pellet in 20  $\mu$ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 10) Load 20  $\mu$ 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 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.
- 12) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 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. x 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. x 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)

