Anti-CRMP2 Antibody [JE31-53]

HA721560



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat
Applications: WB, IF-Cell, FC

Molecular Wt: Predicted band size: 62 kDa

Clone number: JE31-53

Description: Members of the CRMP family were discovered independently in different species by several

groups working in parallel. Among the five members of the family, CRMP-2 was first identified in 1995. Group of researchers led by Goshima found out that CRMP-2 played a role in the transduction of the extracellular Semaphorin 3A (Sema3A), an inhibitory protein for axonal guidance in chick dorsal root ganglion (DRG). The protein was first named as CRMP-62 having a relative molecular mass of 62 kDa and later referred as CRMP-2. Concurrently, a 64 kDa protein named as TOAD-64 for Turned On After Division, was shown to increase significantly during the development of the cortex of the brain. The cDNA sequence of TOAD-64 corresponded to that of rat CRMP-2. In 1996, mouse CRMP-4, often referred to as Ulip for Unc-33 like phosphoprotein, was discovered by Byk and colleagues, using a rabbit polyclonal antiserum which recognized a 64 kDa mouse brain specific phosphoprotein. In the same year, several other studies cloned CRMPs-1-4 in rat and dihydropyrimidinase (DHPase) homologous sequence of CRMPs-1, -2, and -4 in human fetal brain. Finally, in 2000, CRMP-5 was discovered using two-hybrid screenings of brain libraries or purification from a proteic complex. In following researches, CRMPs were studied as target antigens for

autoantibodies in various autoimmune neurodegenerative disorders.

Immunogen: Recombinant protein within Human CRMP2 aa 473-572 / 572.

Positive control: U-87 MG cell lysate, SH-SY5Y cell lysate, Neuro-2a cell lysate, PC-12 cell lysate, mouse

brain tissue lysate, rat brain tissue lysate, SH-SY5Y.

Subcellular location: Cytoplasm. Cytoskeleton.

Database links: SwissProt: Q16555 Human | O08553 Mouse | P47942 Rat

Recommended Dilutions:

WB 1:1,000 IF-Cell 1:100

FC 1:500-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4° C. Store at $+4^{\circ}$ C short term (1-2 weeks). It is recommended to aliquot into

single-use upon delivery. Store at -20 $\ensuremath{^{\circ}}$ long term.

Purity: Protein A affinity purified.

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Images

 Fig1: Western blot analysis of CRMP2 on different lysates with Rabbit anti-CRMP2 antibody (HA721560) at 1/1,000 dilution.

Lane 1: U-87 MG cell lysate (20 µg/Lane) Lane 2: SH-SY5Y cell lysate (10 µg/Lane) Lane 3: Neuro-2a cell lysate (15 µg/Lane) Lane 4: PC-12 cell lysate (10 µg/Lane)

Lane 5: Mouse brain tissue lysate (10 µg/Lane) Lane 6: Rat brain tissue lysate (10 µg/Lane)

Predicted band size: 62 kDa Observed band size: 62 kDa

Exposure time: 5 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721560) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

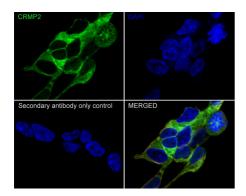


Fig2: Immunocytochemistry analysis of SH-SY5Y cells labeling CRMP2 with Rabbit anti-CRMP2 antibody (HA721560) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CRMP2 antibody (HA721560) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Technical:0086-571-89986345 **Ser**

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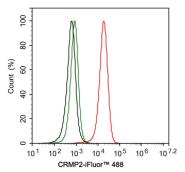


Fig3: Flow cytometric analysis of SH-SY5Y cells labeling CRMP2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721560, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

- 1. Charrier E.; Reibel S.; Rogemond V.; Aguera M.; Thomasset N.; Honnorat J. (August 2003). "Collapsin response mediator proteins (CRMPs) Involvement in nervous system development and adult neurodegenerative disorders". Molecular Neurobiology. 28 (1): 51–63.
- 2. Hou ST, Jiang SX, Smith RA (2008). Permissive and repulsive cues and signaling pathways of axonal outgrowth and regeneration. International Review of Cell and Molecular Biology. Vol. 267. pp. 125–181.