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Product datasheet for AM26484AF-N

SAP155 (SF3B1) Mouse Monoclonal Antibody [Clone ID: 1A5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	1A5
Applications:	WB
Recommended Dilution:	Western blot: 1 µg/ml for chemiluminescence detection system. For details see protocol below. Not recommended for Immunohistochemistry or Immunoprecipitation.
Reactivity:	Hamster, Human, Mouse
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Recombinant mouse Sap155
Specificity:	This antibody reacts with Sap155.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	splicing factor 3b subunit 1
Database Link:	Entrez Gene 23451 Human O75533

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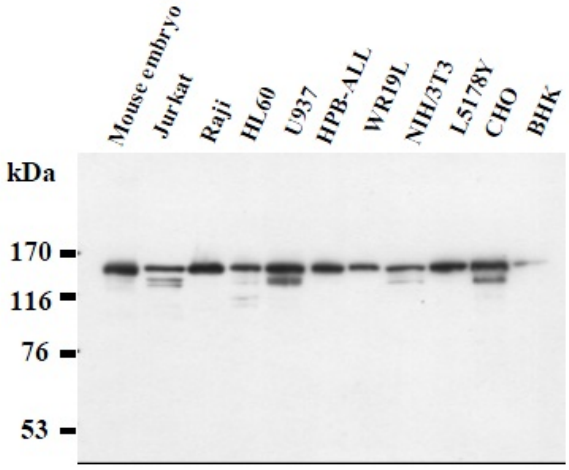
Background: SF3 is a U2 snRNP-associated protein complex essential for spliceosome assembly and splicing catalysis of the major spliceosome. SF3 contains the Spliceosome-Associated Proteins, SAP 49, 130, 145, and 155. SAP155/Sf3b1 is an essential subunit of the U2 snRNP for mRNA splicing and has also been identified in the minor (U12-dependent) spliceosome. SAP155 interacts with the mammalian PcG (Polycomb group) proteins, Mel18 and Ring1B by the yeast two-hybrid system. SAP155 contains numerous Cdk consensus phosphorylation sites in its N terminus and is phosphorylated prior to catalytic step II of the splicing pathway. SAP155 serves as a substrate for cyclin E-cdk2 in vitro, suggesting that pre-mRNA splicing may be linked to the cell cycle machinery in mammalian cells.

Synonyms: Splicing factor 3B subunit 1, SAP-155, SAP 155, SF3b155

Note: This product was originally produced by MBL International.

Protocol:
SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4oC.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 5 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; cell lines)

Product images:



Western blot analysis of Sap155 expression in several cells using AM26484AF-N.