OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26528AF-N

SAP155 (SF3B1) (98-198) Mouse Monoclonal Antibody [Clone ID: 16]

Product data:

Product Type:	Primary Antibodies
Clone Name:	16
Applications:	IF, IP, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 1 µg/200 µl of cell extract from 5x10^6 cells. **Immunocytochemistry:** 10 µg/ml.
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Recombinant GST-Sap155 (98 a.a.- 198 a.a.)
Specificity:	This antibody reacts with Sap155.
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservatives. State: Azide Free State: Liquid purified Ig fraction
Concentration:	lot specific
Purification:	Protein A Agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Predicted Protein Size:	155 kDa
Gene Name:	splicing factor 3b subunit 1
Database Link:	Entrez Gene 23451 Human O75533

View online »

Background: SF3 is a U2 snRNP-associated protein complex essential for spliceosome assembly and splicing catalysis of the majo r 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) sp liceosome. 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 catal ytic 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 machiner y 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 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the 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 2 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 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
8) Wash the membrane with 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 (5 minutes x 6 times).
11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
12) Expose to X-ray film in a dark room for 10 minutes. Develop the film as usual. The conditions for exposure and development may vary.
Positive controls for western blotting: Jurkat, Raji, HeLa, HL60, A431, ZR75-1, Lu99A, WR19L,

L5178Y.

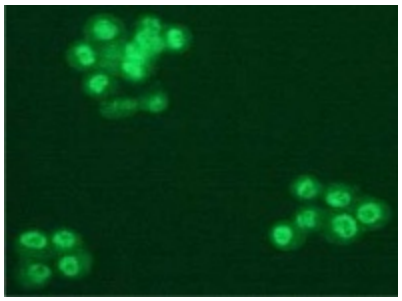
Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 4°C 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 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the APPLICATIONS into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)
Positive control for Immunoprecipitation: Raji.

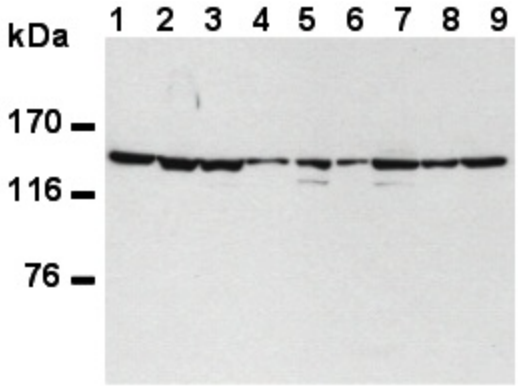
Immunofluorescence microscopy

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1x10e4 cells of HeLa cells for one slide, then incubate in a CO2 incubator for one night.)
2) Fix the cells by immersing the slide in 4% PFA for 20 minutes at room temperature.
3) The glass slides were washed with PBS 3 times.
4) Immerse the slide into PBS containing 0.2% Triton X-100 for 10minutes at room temperature.
5) The glass slide were washed with washing buffer [PBS containing 0.1% Tween20] at 3 times for 5 minutes.
6) Add the primary antibody diluted with blocking buffer as suggested in the APPLICATIONS onto the cells and incubate for 30 minutes at room temperature (Optimization of antibody concentration and incubation conditions are recommended.)
7) The glass slides were washed with washing buffer at 4 times for 5 minutes.
8) Add 100 µL of 1:40 FITC conjugated anti-mouse IgG diluted with blocking buffer onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
9) The glass slides were washed with washing buffer at 3 times for 5 minutes.
10) Wipe excess liquid from the slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.
Positive control for Immunocytochemistry: HeLa.

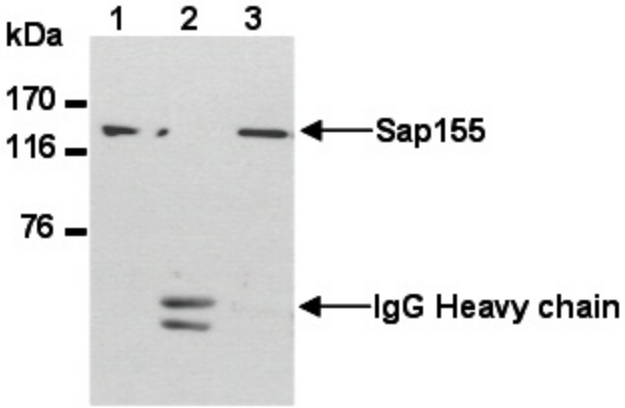
Product images:



Immunocytochemical detection of Sap155 on 4% PFA fixed HeLa cells with AM26528AF-N.



Western blot analysis of Sap155 expression in Jurkat cells (1), Raji cells (2), HeLa cells (3), HL60 cells (4), A431 cells (5), ZR75-1 cells (6), Lu99A cells (7), WR19L cells (8), and L5178Y cells (9) using AM26528AF-N.



Immunoprecipitation of Sap155 from Raji cells with Mouse IgG2b (2) or AM26528AF-N (3). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26528AF-N. Raji cell crude lysate was resolved in lane 1.