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

MONOCLONAL ANTIBODY

Anti-Sap155 mAb

Code No.	Clone	Subclass	Quantity	Concentration
D138-3	1A5	Mouse IgG2b	100 µL	1 mg/mL

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.

SOURCE: This antibody was purified from hybridoma (clone 1A5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant mouse Sap155.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human, mouse and hamster Sap155 on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; Not recommended
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Hamster
Cells and Tissue	Jurkat, Raji, HL60, U937, HPB-ALL	NIH/3T3, WR19L, L5178Y, embryo	CHO, BHK
Reactivity on WB	+	+	+

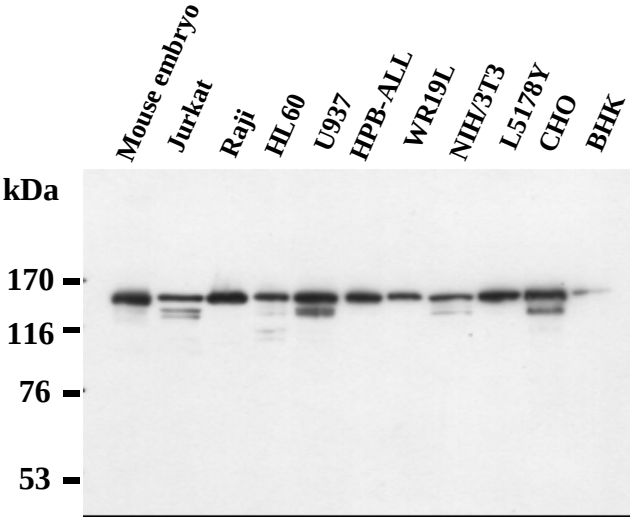
REFERENCES:

1) Atlasi, Y., *et al., PLoS Genet.* **9**, e1003424 (2013) [WB]
2) Eto, K., *et al., Mol. Cell Biochem.* **355**, 217-222 (2011) [WB]
3) Eto, K., *et al., Biochem. Biophys. Res. Commun.* **393**, 577-581 (2010) [WB, IP]
4) Kotake, Y., *et al., Nat. Chem. Biol.* **3**, 570-575 (2007) [WB]
5) Horie, A., *et al., Hybrid. Hybridomics* **22**, 117-119 (2003)

Clone 1A5 is used in these references.

RELATED PRODUCTS:

D221-3	Anti-Sap155 mAb (16)
D139-3	Anti-Ring1B mAb (3-3)
PD043	Anti-Phospho-SF3B1 (Sap155) (Ser129) pAb
RN085PW	Anti-U2AF1 pAb
RN086PW	Anti-U2AF2 pAb
M077-3	Mouse IgG2b (isotype control) (3D12)



Western blot analysis of Sap155 expression in several cells using D138-3.

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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 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. 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/cm^2 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, 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 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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; Jurkat, Raji, HL60, U937, HPB-ALL, mouse embryo, WR19L, NIH/3T3, L5178Y, CHO and BHK)