

MONOCLONAL ANTIBODY

Anti-Sap155 mAb

Code No.	Clone	Subclass	Quantity	Concentration
D221-3	16	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 16) 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 Sap155 (98-198 a.a.).

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 Sap155 (155 kDa) on Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting: 1 µg/mL

Immunoprecipitation: 1 µg/200 µL of cell extract from 5x10⁶ cells

Immunohistochemistry: Not tested

Immunocytochemistry: 10 µg/mL

Flow cytometry: Not tested

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

INTENDED USE:

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

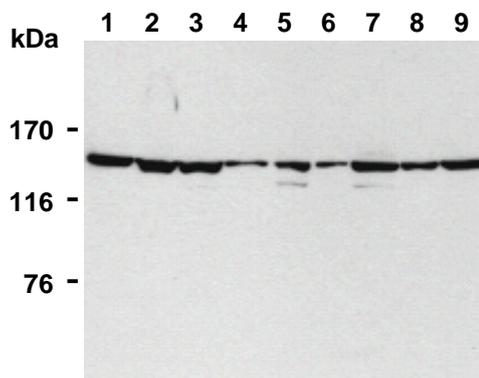
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, HeLa, Raji, HL-60, A431, ZR-75-1, Lu99A	WR19L, L5178Y	Not tested
Reactivity on WB	+	+	

REFERENCES:

- 1) Kfir, N., *et al.*, *Cell Rep.* **11**, 618-629 (2015) [WB, IP]
- 2) Schreiber, C. A., *et al.*, *PLoS Pathog.* **11**, e1005082 (2015) [WB]
- 3) Gao, Y., *et al.*, *Sci. Rep.* **4**, 6098 (2014) [WB]
- 4) Kajiwara, T., *et al.*, *Cancer Sci.* **104**, 149-156 (2013) [WB]
- 5) Manceau, V., *et al.*, *PLoS One* **7**, e43946 (2012) [WB]
- 6) Takemura, R., *et al.*, *Genes Cells.* **16**, 1035-1049 (2011) [WB]
- 7) Yokoi, A., *et al.*, *FEBS J.* **278**, 4870-4880 (2011) [WB]
- 8) Tanuma, N., *et al.*, *J. Biol. Chem.* **283**, 35805-35814 (2008) [WB, IP]
- 9) Yamasaki, S., *et al.*, *Nat. Immunol.* **9**, 1179-1188 (2008) [WB]
- 10) Kotake, Y., *et al.*, *Nat. Chem. Biol.* **3**, 570-575 (2007) [WB, IP]
- 11) Horie, A., *et al.*, *Hybrid Hybridomics* **22**, 117-119 (2005)

Clone 16 is used in these references.



Western blotting analysis of Sap155 expression in Jurkat (1), Raji (2), HeLa (3), HL-60 (4), A431 (5), ZR-75-1 (6), Lu99A (7), WR19L (8) and L5178Y (9) using D221-3

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

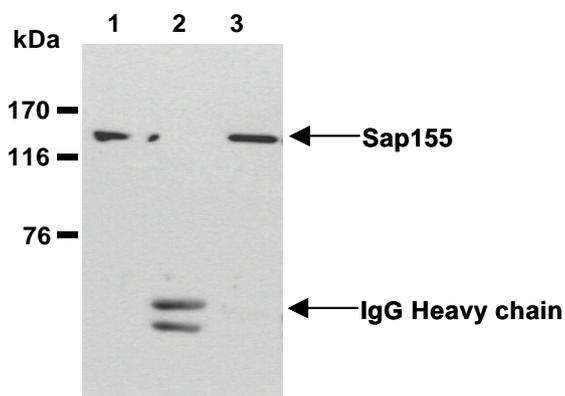
SDS-PAGE & Western blotting

- 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 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% methanol). 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 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6).
- 9) Incubate the membrane with 1:10,000 of 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).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, HeLa, HL-60, A431, ZR-75-1, Lu99A, WR19L and L5178Y)

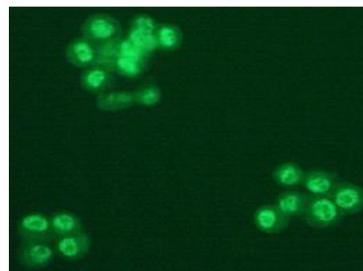


Immunoprecipitation of Sap155 from Raji with Mouse IgG2b (2) or D221-3 (3). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D221-3. Raji cell crude lysate was resolved in lane 1.

Immunoprecipitation

- 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.
- 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.
- 4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 5) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
- 6) Resuspend the beads with cold Lysis buffer.
- 7) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
- 8) Repeat steps 6)-7) 3-5 times.
- 9) 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)



Immunocytochemical detection of Sap155 on 4% PFA fixed HeLa with D221-3.

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10⁴ cells for one slide, then incubate in a CO₂ incubator overnight.)
- 2) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 20 minutes at room temperature.
- 3) The glass slide was washed 3 times with PBS.
- 4) Immerse the slide into PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
- 5) The glass slide was washed with washing buffer [PBS containing 0.1% Tween-20] at 3 times for 5 minutes.
- 6) Add the primary antibody diluted with washing buffer as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 7) The glass slide was washed as in the step 5).

- 8) Add FITC conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 9) The glass slide was washed as in the step 5).
- 10) Wipe excess liquid from 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)

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