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Not for use in diagnostic procedures.**

MBL
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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 $5x10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; 10 µg/mL
Flow cytometry; Not tested

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

INTENDED USE:

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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:

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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]
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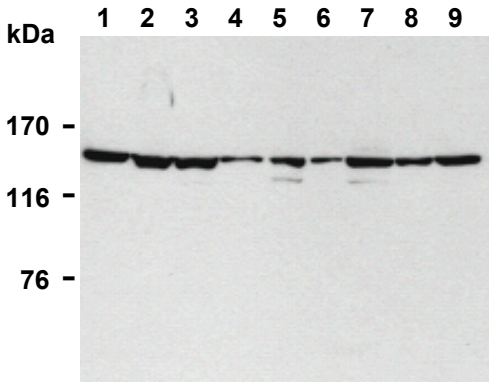
7) Yokoi, A., *et al*., *FEBS J*. **278**, 4870-4880 (2011) [WB]
8) Tanuma, N., *et al*., *J. Biol. Chem*. **283**, 35805-35814 (2008) [WB, IP]
9) Yamasaki, S., *et al., Nat. Immunol*. **9**, 1179-1188 (2008) [WB]
10) Kotake, Y., *et al., Nat. Chem. Biol*. **3**, 570-575 (2007) [WB, IP]
11) Horie, A., *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.

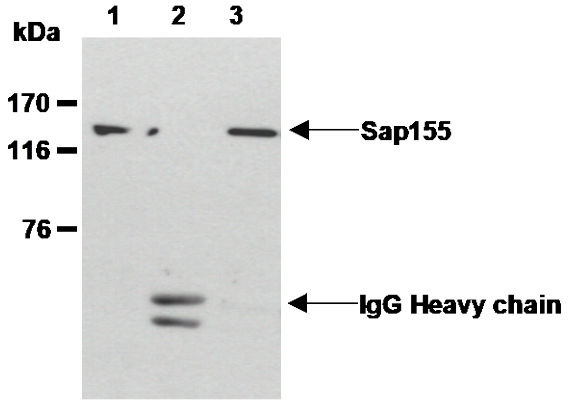
PROTOCOLS:

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/cm^2 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)

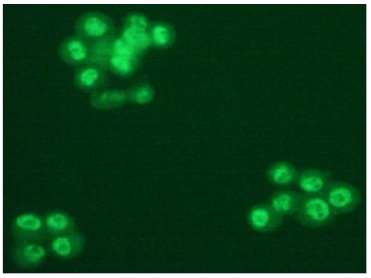


Immunoprecipitation of Sap155 from Raji with Mouse IgG2b (2) or D221-3 (3). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D221-3. Raji cell crude lysate was resolved in lane 1.

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 10^4 cells for one slide, then incubate in a CO_2 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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