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

Anti-DDX21 pAb

CODE No. RN090PW

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified Ig from rabbit serum
FORMULATION 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.

APPLICATIONS-CONFIRMED

Western blotting 1:1,000
Immunoprecipitation 5 µL/500 µL of cell extract from 2 x 10^7 cells

APPLICATIONS-UNDER EVALUATION

Immunocytochemistry 1:400

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, A431, 293T	Not tested	Not tested	Not tested
Reactivity	+			

Entrez Gene ID 9188 (Human)

For more information, please visit our web site **https://ruo.mbl.co.jp/je/rip-assay/**.

RELATED PRODUCTS

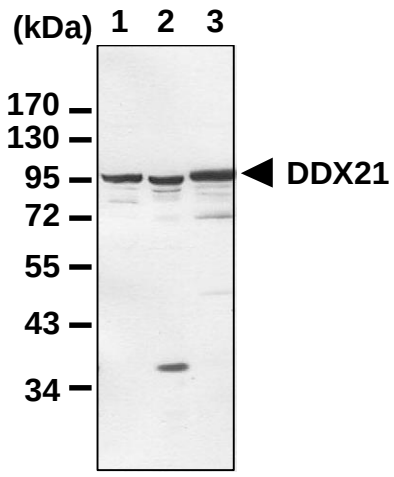
Please visit our web site https://ruo.mbl.co.jp/.

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

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for overnight at 4ºC.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (10 min. x 3).
8) Incubate the membrane with the 1:5,000 anti-rabbit IgG-HRP (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (10 min. x 3).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T and A431)

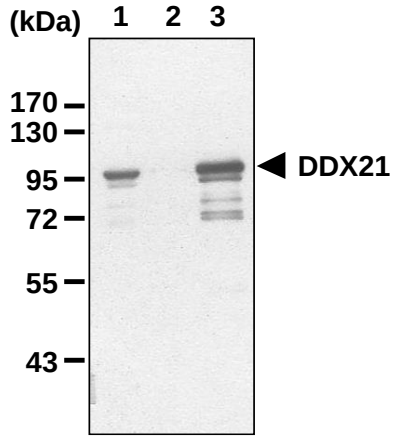


Western blot analysis of DDX21
Lane 1: HeLa
Lane 2: 293T
Lane 3: A431
Immunoblotted with RN090PW

Immunoprecipitation

1) Wash 4 x 10^7 cells twice with PBS and resuspend them with 1 mL of ice-cold Lysis buffer [150 mM NaCl, 20 mM Tris-HCl (pH 8.0), 0.1% NP-40, 10 mM EDTA] containing appropriate protease inhibitors and 1.5 mM DTT. Vortex thoroughly, then incubate it on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4ºC and discard the supernatant.
3) Wash the pellet 3 times with PBS and resuspend them with 500 µL RIPA buffer, then sonicate briefly.
4) Centrifuge the tube at 12,000 x g for 5 min. at 4ºC and transfer the supernatant to another fresh tube.
5) Add 500 µL of ice-cold Lysis buffer into the supernatant. Mix by pipetting up and down.
6) Add 40 µL of 50% protein G agarose beads slurry resuspended in Lysis Buffer into the sample (prepared from step 5). Incubate it at 4ºC with rotating for 1 hour.
7) Centrifuge the tube at 2,000 x g for 2 minutes at 4ºC and transfer the supernatant to another tube (precleared sample).
8) Mix 20 µL of 50% protein G agarose beads slurry resuspended in PBS with normal rabbit IgG (RIP-Assay Kit) or anti-DDX21 pAb at the amount suggested in the **APPLICATIONS,** then add 1 mL of Lysis Buffer into each tube. Incubate with gentle agitation for 1 hr. at 4ºC.
9) Wash the beads once with 500 µL of ice-cold Lysis Buffer (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipette or without disturbing the beads.
10) Add 500 µL of nuclear extract (the sample from step 7), then incubate with gentle agitation for 3 hr. at 4ºC.
11) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 min.).
12) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min., and centrifuge for 5 min. Use 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
13) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
14) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature, or overnight at 4ºC.
15) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
16) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
17) Wash the membrane with PBS-T (10 min. x 3).
18) Incubate the membrane with the 1:1,000 Rabbit TrueBlot® anti-Rabbit IgG-HRP (eBioscience, code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
19) Wash the membrane with PBS-T (10 min. x 3).
20) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
21) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

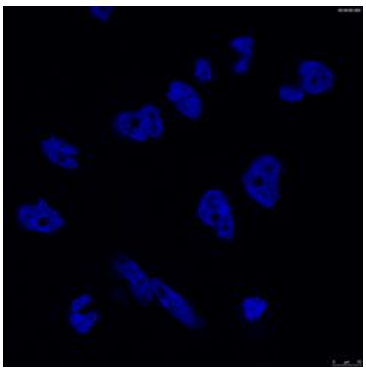
(Positive control for Immunoprecipitation; HeLa nuclear extract)

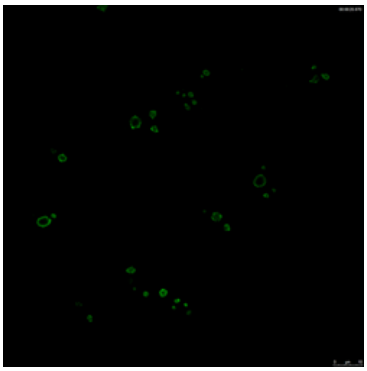


Immunoprecipitation of DDX21 from HeLa
Lane 1: Input
Lane 1: IP with normal rabbit IgG
Lane 2: IP with RN090PW
Immunoblotted with RN090PW

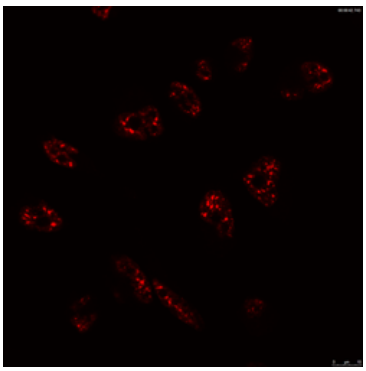
Immunocytochemistry (Under evaluation)

DAPI | **DDX21**

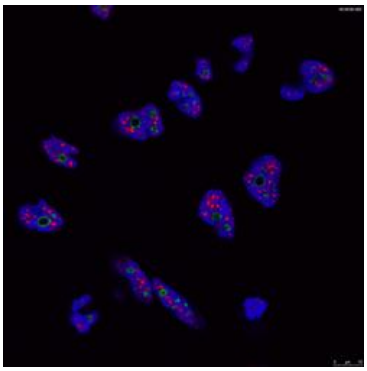




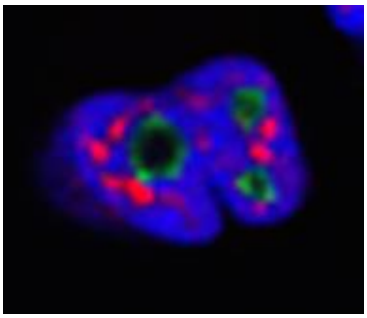
SC35
(Nuclear speckle marker)



Merge



Merge (enlarged)



Immunocytochemical detection of DDX21 in HeLa Tet-Off cell

These data were provided by Dr. Akimitsu, The University of Tokyo.