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Polyclonal Antibody to CDC14A2 - Aff - Purified

Catalog No.: 15-288-21521

Quantity: 0.1 mg

Concentration: 1.0 mg/ml

Background: The hnRNPs belong to a subfamily of ubiquitously expressed heterogeneous nuclear ribonucleoproteins. hnRNPs are RNA binding proteins and they complex with heterogeneous nuclear RNA (hnRNA). These proteins are associated with pre-mRNAs in the nucleus and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport. While all of the hnRNPs are present in the nucleus, some seem to shuttle between the nucleus and the cytoplasm. The HNRNP proteins have distinct nucleic acid binding properties.

Host / Isotype: Chicken

Format: **State:** Liquid purified Ig faction.
Purification: Protein A chromatography.
Buffer System: PBS containing 0.09% Sodium Azide as preservative.

Applications: ELISA.
Western blot (1/1,000), detects a band of approximately 53/56 kDa.
Immunoprecipitation.
Immunofluorescence.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The antibody recognizes human hnRNP-F/H.
Cross reacts with Xenopus.

Storage: Store the antibody at 2-8°C for one month, or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: One year from despatch.

Protocols: **Immunofluorescence protocol - Formaldehyde fixation**
1. Collect cells from T.c.unit and remove media from petri dish using suction.
2. Wash with 1x PBS and remove.
3. Incubate cells in pre-warm (37°C) Para-Formaldehyde for 12 minutes at room temperature on an orbital shaker.
4. Remove PFA and incubate in 0.5% Triton X-IOO in 1x PBS for 5 minutes at room temperature.
5. Prepare blocking reagent, this is also the antibody diluent.
6. Wash cells 2x with 1x PBS at room temperature, for 4 minutes/wash on an orbital shaker.
7. Block with 1 % NCS and 1x PBS for 30 minutes at room temperature.
8. Prepare primary antibodies (50µl/coverslip) and moist staining chambers.

9. Wash cells 2x with lx PBS at room temperature and air dry briefly.
10. Incubate with primary antibody for 1 hr at room temperature in the dark in staining chambers. During this time prepare the secondary antibody.
11. Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
12. Incubate with secondary antibody for 1 hour at room temperature in the dark in staining chambers.
13. Wash cells 5x with 1x PBS.
14. Mount in Dapi.

Solutions (prepare fresh the same day of staining).
1x Phosphate buffered saline.
Blocking reagent: 1% NCS in 1x PBS (use fresh l0x PBS).
Fixation solution: 3.5% Para formaldehyde.
1.75g PFA in 20 ml d.H20 plus 5 drops 1M NaOH. Stir on a hot plate at 50-60°C until dissolved. Add 4 drops I N HCI and check pH indicator strip. pH should be 7.4. Complete volume with d.H20 to 25ml and add 25ml 2xPBS. Check pH before adding to cover slips.

Immunofluorescence protocol - Methanol/acetone fixation
1. Collect cells from T.C.unit and remove media from petri dish using suction.
2. Wash with 1x PBS and remove.
3. Fix cells with cold methanol: acetone 1: 1 for 10 minutes on ice.
4. Prepare blocking reagent, this is also the diluent for the antibodies.
5. Remove fixative and wash cells 3x with Ix PBS at RT, for 4 minutes/wash on orbital shaker.
6. Block with 1% NCS and Ix PBS for 30 minutes at RT.
7. Prepare primary antibodies (50µl/coverslip) and moist staining chambers.
8. Wash cells 2x with 1 x PBS at RT and air dry for approximately 7 minutes.
9. Incubate with primary antibody for 1 hr at RT in the dark in staining chambers. During this time prepare secondary antibody.
10. Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
11. Incubate with secondary antibody for 1 hr at R T in the dark in staining chambers.
12. Wash cells 5x with 1x PBS.
13. Mount in Dapi.

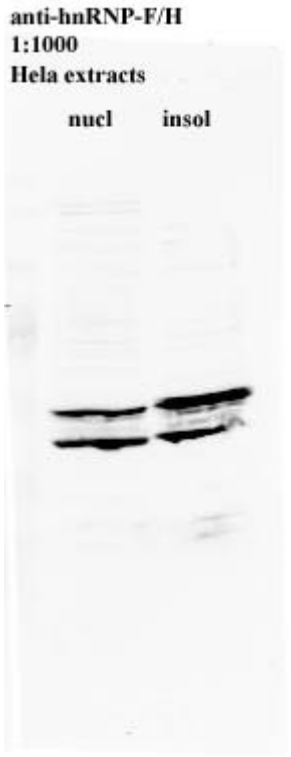
Solutions (prepare fresh the same day of staining)
1x Phosphate buffered saline.
Blocking reagent: 1% NCS in 1x PBS (use fresh 10x PBS).
Fixation solution: methanol:acetone 1: 1 ice cold.

Western Blotting Protocol
1. Transfer gel to PDVF or nitrocellulose membrane
2. Place membrane in plastic tray in blocking buffer for one hour with agitation
3. Rinse in wash buffer
4. Incubate in wash buffer plus primary antibody for one hour
5. Wash 6 X 5 minutes with wash buffer
6. Incubate in wash buffer plus secondary antibody for one hour
7. Wash 6X 5 minutes with wash buffer
8. Detect (e.g. ECL, Amersham according to manufacturers instructions)

Wash buffer

PBS + 0.1% Tween 20
Blocking buffer
Wash buffer + 5% dried milk powder

Pictures:



Western blot of anti-hnRNP F/H on HeLa cell extract using cat.no. BP4547P