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Polyclonal Antibody to NUP153 - Aff - Purified

Alternate names: 153 kDa nucleoporin, Nuclear pore complex protein Nup153, Nucleoporin Nup153

Catalog No.: BP4506

Quantity: 2 ml

Background: Nuclear pore complexes are extremely elaborate structures that mediate the regulated movement of macromolecules between the nucleus and cytoplasm. These complexes are composed of at least 100 different polypeptide subunits, many of which belong to the nucleoporin family. Nucleoporins are pore complex specific glycoproteins characterized by cytoplasmically oriented O linked N acetylglucosamine residues and numerous repeats of the pentapeptide sequence XFXFG. Nup153 has three distinct domains: a N terminal region within which a pore targeting domain has been identified, a central region containing multiple zinc finger motifs, and a C terminal region containing multiple XFXFG repeats. Nup153 is a possible DNA binding subunit of the nuclear pore complex (NPC). The repeat containing domain may be involved in anchoring components of the pore complex to the pore membrane.

Uniprot ID: P49790

NCBI: NP_005115.2

GeneID: 9972

Host: Sheep

Immunogen: Synthetic peptide from a region of Human Nup153 (residues 1456-1475).

Format:
State: Liquid purified Ig fraction.
Purification: Affinity Chromatography.
Buffer System: PBS with 0.09% Sodium Azide as preservative.

Applications: Suitable for Immunoprecipitation, Immunofluorescence (neat) and Western blot (1/10). Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Detects recombinant and endogenous Nup153 in Human, Rat and Xenopus.

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

General Readings:
1. Smythe, C. et al., The Embo Journal 2000, 19 (15), 3918-3931.

Protocols:
Immunofluorescence protocol - Formaldehyde fixation
Collect cells from T.c.unit and remove media from petri dish using suction.
Wash with 1x PBS and remove.
Incubate cells in pre-warm (37°C) Para-Formaldehyde for 12 minutes at room temperature on an orbital shaker.

Remove PFA and incubate in 0.5% Triton X-IOO in 1x PBS for 5 minutes at room temperature.
Prepare blocking reagent, this is also the antibody diluent.
Wash cells 2x with 1x PBS at room temperature, for 4 minutes/wash on an orbital shaker.
Block with 1 % NCS and 1x PBS for 30 minutes at room temperature.
Prepare primary antibodies (50µl/coverslip) and moist staining chambers.
Wash cells 2x with lx PBS at room temperature and air dry briefly.
Incubate with primary antibody for 1 hr at room temperature in the dark in staining chambers. During this time prepare the secondary antibody.
Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
Incubate with secondary antibody for 1 hour at room temperature in the dark in staining chambers.
Wash cells 5x with 1x PBS.
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 IN 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

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

Transfer gel to PDVF or nitrocellulose membrane
Place membrane in plastic tray in blocking buffer for one hour with agitation
Rinse in wash buffer
Incubate in wash buffer plus primary antibody for one hour
Wash 6 X 5 minutes with wash buffer
Incubate in wash buffer plus secondary antibody for one hour
Wash 6X 5 minutes with wash buffer
Detect (e.g. ECL, Amersham according to manufacturers instructions)
Wash buffer: PBS + 0.1% Tween 20
Blocking buffer: Wash buffer + 5% dried milk powder
The concentration of antibodies used depends on each antibody, the amount of antigen and the detection method used. Generally, dilution is in the range of a few hundred times

dilution to a few thousand times dilution, but usually has to be determined empirically.

Pictures:

Confocal Microscope analysis of Nup153, Lamin B3 and F/GXFG nucleoporin distribution in sperm pronuclei.