

Polyclonal Antibody to LysoH - Aff - Purified

Catalog No.:	15-288-21066
Quantity:	0.1 mg
Host / Isotype:	Chicken
Immunogen:	Purified recombinant GST-Nup53p.
Format:	State: Liquid antiserum with 0.09% sodium azide as preservative.
Applications:	Western blot. Immunofluorescence. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	Yeast Nup53p.
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 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-100 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 1x 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. <u>Solutions (prepare fresh the same day of staining):</u> 1x Phosphate buffered saline. Blocking reagent: 1% NCS in 1x PBS (use fresh 10x PBS). Fixation solution: 3.5% Para formaldehyde. 1.75g PFA in 20 ml d.H2O plus 5 drops 1M NaOH. Stir on a hot plate at 50-60°C until

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dissolved. Add 4 drops IN HCl and check pH indicator strip. PH should be 7.4. Complete volume with d.H2O 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 1x PBS at RT, for 4 minutes/wash on orbital shaker.

Block with 1% NCS and 1x 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.