

Immunocytochemistry

Reagents:

- 1) AKIN13
- 2) Rhodamine conjugated anti-rabbit antibody (Cat. # RG05)
- 3) Phosphate Buffered Saline (PBS)
- 4) 100% methanol (-20°C)
- 5) Blocking solution (PBS plus 3% BSA)
- 6) 100 nM DAPI (4',6-diamidino-2-phenylindole) in PBS
- 7) Antifade mounting medium (Molecular Probes Inc. Cat.# S-7461)
- 8) Glass microscope slide (25 x 75 x 1 mm)
- 9) Coverslip sealing solution (clear nail polish)

Method:

- 1) Grow tissue culture cells on glass coverslips until semi-confluent.
- 2) Remove culture media and gently wash the cells once with PBS at 37°C.
- 3) Fix the cells with 100% methanol at -20°C for 3 min (all remaining steps are at room temperature).
- 4) Wash the cells once with PBS at room temperature for 30 sec.
- 5) Place the coverslips on parafilm, and block with 3% BSA in PBS for 1 h in a humid atmosphere.
- 6) Wash the coverslips once with PBS.
- 7) Add 200 µl of 1 µg/ml (1:250 dilution) of AKIN13 antibody in blocking solution to each coverslip. Incubate for 1 h.
- 8) Wash each coverslip three times in PBS plus 1% Triton X-100 (let stand for 5 min each).
- 9) Add 200 µl of a 1:500 dilution of rhodamine conjugated anti-rabbit antibody (Cat. # RG05) in blocking solution to each coverslip. Incubate for 30 min.
- 10) Wash each coverslip three times in PBS (let stand for 5 min each).
- 11) Counterstain the DNA for 5 min with 200 µl of 100 nM DAPI in PBS.
- 12) Invert the coverslips on a drop of antifade mounting media on a glass slide. Gently remove the excess media with a tissue and seal each side with nail polish.
- 13) Store the slides in the dark at 4°C.
- 14) Typical results are shown in Figure 4 and are similar to published data (Sueishi et al., 2000; J Biol Chem 275:28888-28892).

Figure 4. Immunofluorescence images of HeLa cells stained with AKIN13 antibody. Cells were grown to semi-confluency and fixed with methanol. Immunofluorescence staining of Hk1p2 (red) in HeLa cells is shown using 1 µg/ml (1:250 dilution) of AKIN13 antibody. Primary antibody was detected with a 1:500 dilution of goat anti-rabbit rhodamine conjugated antibody (Cat. # RG05). DNA (blue) was stained with 100 nM DAPI in PBS. Photograph taken with a 100X objective lens.



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Anti-Hk1p2 (rabbit origin)

Cat. # AKIN13

Lot # 011 Amount: 1 x 50 µg

Shipped at room temperature

Store lyophilized powder at 4°C

Material

AKIN13 is provided as an affinity purified rabbit polyclonal antibody. The antibody was raised against the tail region of human kinesin Hk1p2 (KIF15, KNSL7). Native Hk1p2 has a predicted molecular weight of 160 kDa. Proteolytic breakdown products of Hk1p2 (e.g. 55 kDa) may be present in Western blots of some cell extracts. A HeLa cell extract is included as a positive control for antibody reactivity (see Fig. 1). AKIN13 (50 µg of protein) is supplied as a lyophilized white powder.

Storage and Handling

Upon receipt, AKIN13 should be briefly centrifuged to collect the white powder at the bottom of the tube and stored desiccated at 4°C. Reconstitute the antibody to 250 µg/ml by resuspending in 200 µl of Milli-Q water plus 30% glycerol and store at 4°C for up to one month. For storage longer than one month the antibody should be aliquoted, snap frozen in liquid nitrogen and stored at -70°C. Resuspend the positive control protein in 100 µl of 10% glycerol in Milli-Q water for a final concentration of 2 mg/ml, aliquot into 10 x 10 µl amounts (20 µg each), freeze in liquid nitrogen and store at -70°C. There is sufficient HeLa extract for 10 positive controls.

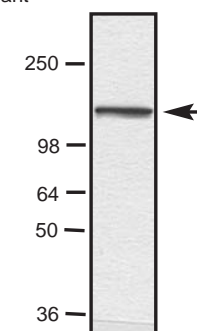
Product Uses

This antibody is recommended for detection of human klp2, which is abundant in dividing cells. AKIN13 has been tested with extracts from other species, and klp2 was detected in mouse and rat cell extracts (Fig. 2).

The following protocols have been tested with this antibody:

- * Western blot analysis: recommended
- * Immunocytochemistry: recommended
- * Immunoprecipitation: recommended

Figure 1. Western blot analysis of anti-Hk1p2 antibody. Protein samples were separated by electrophoresis and transferred to PVDF membrane as described in the methods. AKIN13 antibody was diluted to 500 ng/ml (1:500) for Western blot analysis. Hk1p2 was detected in 20 µg of HeLa cell extract (see arrow). Molecular weight markers are from Invitrogen.



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Methods

Western blot analysis

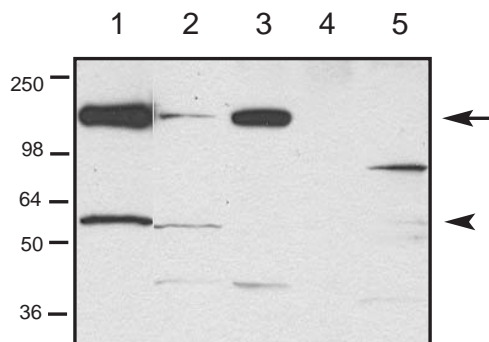
Reagents:

- 1) AKIN13
- 2) SDS-PAGE and Western blot apparatus
- 3) PVDF or Nitrocellulose membrane (Millipore Inc)
- 4) TBST: 20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.01% Tween 20
- 5) HRP goat anti-rabbit antibody (Cat. # RG04)
- 6) Chemiluminescence detection reagents (Pierce SuperSignal West Dura Cat. # 34075)

Method:

- 1) Separate protein samples on a 12% SDS PAGE gel until the dye-front reaches the bottom of the gel.
- 2) Electroblot the proteins onto PVDF or nitrocellulose membrane for 60 min using 350 mA with fresh transfer buffer (25 mM Tris-HCl, pH 8.3; 192 mM glycine, 5% methanol).
- 3) Block the membrane in TBST + 5% non-fat dry milk for 30 min at room temperature. (All remaining steps are at room temperature).
- 4) Probe with 500 ng/ml (1:500 dilution) of AKIN13 antibody in TBST + 5% non-fat dry milk for 1 h. A 1:250 dilution of antibody can be used if protein samples have a low abundance of Hklp2.
- 5) Wash the membrane three times with TBST for 10 min each.
- 6) Probe with 1:40,000 HRP anti-rabbit in TBST + 5% non-fat dry milk for 1 h. A 1:25,000 dilution can be used if protein samples have a low abundance of Hklp2.
- 7) Wash the membrane six times with TBST for 10 min each.
- 8) Process the blots for chemiluminescence detection.
- 9) Typical assay results are shown in Figures 1 and 2.

Figure 2. Western blot of cell extracts probed with AKIN13 antibody. Chemiluminescence detection of Hklp2 in 20 µg HeLa cell extracts (lane 1, arrow; breakdown product at 55 kDa shown as arrow head). Other lanes shown on the blot are mouse 3T3 (lane 2), rat NRK (lane 3), drosophila S2 (lane 4), and xenopus A6 (lane 5) cell extracts (50 µg each). (Lane 1 was from another region of the gel).



Immunoprecipitation

Reagents:

- 1) AKIN13
- 2) Non-immune rabbit IgG (control antibody).
- 3) Immunoprecipitation (IP) Buffer (4°C): 50 ml of 150 mM NaCl, 1% NP-40, 50 mM PIPES pH7.0, 1mM PMSF (made fresh).
- 4) Protein A beads (Amersham Biosciences Cat.# 17-5280-01): 300 µl 50% bead slurry in IP Buffer. Wash beads 3x in 5 ml H₂O and 3x in 5 ml IP Buffer. Resuspend to 50% slurry in IP Buffer.
- 5) Phosphate buffered saline (PBS): 50 ml at 37°C.
- 6) Cell lysates from three 9 cm plates of human tissue culture cells (e.g. HeLa), mid exponential cells. Wash cells with PBS. Pipette 1 ml of IP Buffer into the first plate and scrape off the cells. Transfer buffer to the second and third plates and scrape. Pool samples and place on ice. Observe cells for lysis under a microscope. If cells are not lysed, sonicate 5 sec on ice and observe for cell lysis. Repeat sonication if necessary. Microfuge lysate at 4°C for 10 min at 14,000 xg. Pipette supernatant into a new tube on ice. Assay protein content of supernatant, and aliquot 500 µg into two 1.5 ml tubes (labeled AKIN13 and CONTROL).

Method:

- 1) Dilute the lysate samples to 1.0 mg/ml with IP Buffer. Take 20 µl of the lysate from each tube for Western blot analysis and add SDS sample loading buffer (1x final) to the 20 µl sample.
- 2) Preclear the lysate with Protein A beads. Pipette 50 µl of a 50% Protein A slurry into the AKIN13 and CONTROL tubes and incubate on a rotator at 4°C for 1 h.
- 3) Spin down the beads for 2 min in a microfuge at 14,000 xg at 4°C.
- 4) Pipette supernatant into new tubes and add 2 µg of the AKIN13 antibody (AKIN13 tube) and 2 µg control rabbit IgG (CONTROL tube). Incubate on a rotator at 4°C for 1 h.
- 5) Add 50 µl of a 50% Protein A slurry to each tube. Incubate on a rotator at 4°C for 1 h.
- 6) Spin down the beads for 2 min in a microfuge at 14,000 xg at 4°C. Remove supernatant and add SDS sample loading buffer (2x final with fresh dithiothreitol) to a 100 µl sample of the supernatant.
- 7) Wash beads 3 times in 1 ml IP Buffer. Finally, resuspend beads in 50 µl 4x SDS sample loading buffer that contains fresh dithiothreitol. Boil samples for 5 min at 100°C.
- 8) Run a SDS-PAGE gel with 20 µl each of the following samples: lysate, IP supernatant, IP beads, and control beads. Blot the gel to PVDF membrane and perform Western blot analysis by probing with 500 ng/ml AKIN13 antibody (1:500 dilution).
- 9) Typical results are shown in Figure 3.

Figure 3. Immunoprecipitation of human Hklp2 from HeLa extracts using AKIN13. Western blot of HeLa cell lysate (Lane 1), IP supernatant after immunoprecipitation (Lane 2), Hklp2 immunoprecipitated with AKIN13 (160 kDa, see arrow) eluted from Protein A beads (lane 3), and control immunoprecipitation reaction with normal rabbit IgG (lane 4). Lanes 3 and 4 also show IgG heavy chain (55 kDa, arrow head). The Western blot was probed with 500 ng/ml (1:500 dilution) of AKIN13 antibody.

