

ORDERING INFORMATION

Catalog Number: AF4768

Lot Number: CAVL01

Size: 100 µg

Specificity: human PIAS2

Immunogen: *E. coli*-derived rhPIAS2
(aa 1 - 550)

Ig Type: sheep IgG

Application: Western blot

Background

PIAS2 (Protein inhibitor of activated STAT2; also DIP, MIZ1 and PIASx-β) is a 45 - 70 kDa member of the PIAS family of molecules. In primary cells and tissues it is expressed in T and NK cells, and in testis where it likely regulates spermatogenesis. However, it is widely expressed in cultured cell lines. PIAS2 is known to inhibit STAT4-mediated gene activation, and to interact with inactive ELK-1, promoting its activation via HDAC2 dissociation and desumoylation. Human PIAS2 is 621 amino acids (aa) in length. It contains a SAP domain (aa 11 - 45), a zinc-finger region (aa 331 - 408), a SUMO-binding motif (aa 467 - 472) and an NLS (aa 484 - 492). There are multiple splice variants. PIAS2x-β/ARIP3 shows a 22 aa substitution for aa 551 - 621, while PIAS-NY shows a 12 aa substitution for aa 1 - 8, followed by a truncation after Gly401. Two others show a four aa substitution for aa 504 - 621, and an alternate start site at Met10. Over aa 1 - 550, human PIAS2 shares 98% aa identity with mouse PIAS2.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived recombinant human PIAS2 (rhPIAS2; aa 1 - 550; Accession # O75928). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% Na₃N.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human PIAS2 in Western blot with an approximate molecular weight of 63 - 70 kDa.

Application

Western blot - An antibody concentration of 0.1 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween® 20

Blocking Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

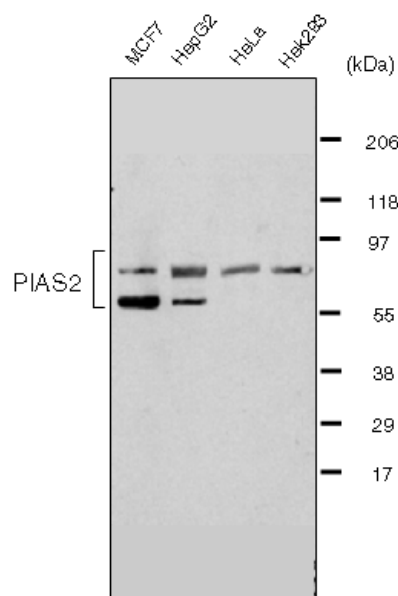
Antibody Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane for 1 hour room temperature in Antibody Solution containing 0.1 µg/mL sheep anti-human PIAS2.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:5000 dilution of HRP-conjugated donkey anti-sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - A single plate (150 mm) of exponentially growing cells is washed twice in cold PBS. 1 mL of boiling 1% SDS lysis buffer (1% SDS, 10 mM Tris-HCl, pH 7.4, 1 mM sodium ortho-vanadate) is added to the plate. The plate is then scraped and the lysis is collected, sonicated and quantified. 30 µg of cellular protein is added to an equal amount of 2x SDS loading buffer. Samples are then boiled for 5 minutes and run on a SDS-PAGE gel.

Optimal dilutions should be determined by each laboratory for each application.



Detection of PIAS2 with AF4768.

30 µg of whole cell extracts from exponentially growing MCF7, HepG2, HeLa, and Hek293 cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 0.1 µg/mL sheep anti-PIAS2 antibody, as described in *Protocols for Immunoblotting*.