Affinity-purified Sheep Anti-human/mouse ZNF281 Antibody

ORDERING INFORMATION

Catalog Number: AF6350

Lot Number: CDST01

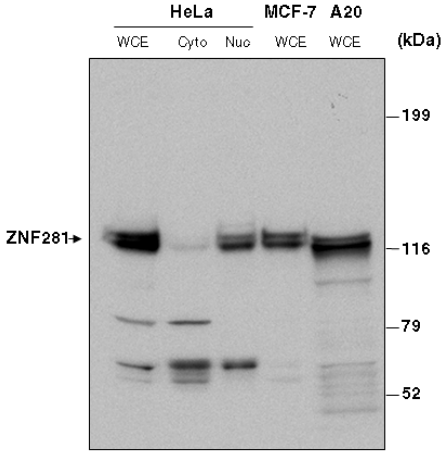
Size: 100 μg

Specificity: human and mouse ZNF281

Immunogen: *E. coli*-derived rhZNF281 (aa 681 - 895)

Ig Type: sheep IgG

Application: Western blot



Detection of ZNF281 with AF6350. Lysates from human HeLa whole cell extracts (WCE), Cytoplasmic (Cyto) and Nuclear (Nuc) fractions, as well as WCE from human MCF7 and mouse A20 cells were resolved by SDS-PAGE. Following electrophoresis, lysates were transferred to a PVDF membrane and immunoblotted with 1.0 μg/mL anti-ZNF281, as described in *Protocols for Immunoblotting*.

Background

ZNF281 (zinc finger protein 281; also ZBP99 and GZP1) is a 100 kDa member of the Krueppel C2H2-type Zn-finger family of proteins. It is found in embryonic stem cells, placenta, and lymphocytes, and appears to be necessary for the maintenance of pluripotency in stem cells. ZNF281 both represses (STAT3) and activates (Nanog) multiple genes, and is known to form a complex with Nanog, Oct4 and Sox2 . Human ZFN281 is 895 amino acids in length. It contains one Gly-rich region (aa 4 - 37), a poly-Pro motif (aa 90 - 96) and four consecutive C2H2-type zinc finger domains (aa 261 - 367). There are phosphorylation sites at Ser255, 395, 484, 785 and 807. Potential alternate start sites exist at Met296 and Met434. Over aa 681 - 895, human ZFN281 shares 99% aa identity with mouse ZFN281.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived recombinant human ZNF281 (rhZNF281; aa 681 - 895; Accession # Q9Y2X9). 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% NaN_3.

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 and mouse ZNF281 in Western blot with an approximate molecular weight of 116 kDa.

Application

Western blot - An antibody concentration of 1.0 μg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to a PVDF membrane 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 1.0 μg/mL sheep anti-human/mouse ZNF281.
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 1:5,000 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.

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