

# Affinity-purified Sheep Anti-human/mouse ZIC3 Antibody

## ORDERING INFORMATION

**Catalog Number:** AF5310

**Lot Number:** CBVV01

**Size:** 100 µg (sufficient for 50 mL of blotting solution)

**Storage:** -20° C

**Specificity:** human/mouse ZIC3

**Immunogen:** *E. coli*-derived rhZIC3 (aa 380 - 467)

**Ig Type:** sheep IgG

**Application:** Western blot

## Background

ZIC3 (zinc-finger protein of the cerebellum 3) is a 50 kDa (predicted) member of the GLI C2H2-type Zn-finger protein family. It is expressed in cerebellum, and apparently plays a key role in left-right body axis formation. Human ZIC3 is 467 amino acids (aa) in length. It contains N-terminal poly-Ala (aa 46 - 55) and poly-His (aa 87 - 97) segments, followed by five C2H2-type Zn-fingers (aa 253 - 410). Within the zinc finger region, there is an overlapping NES (aa 290 - 349) and two NLS's (aa 367 - 382 and 403 - 410). ZIC3 reportedly binds to GLI-3. There is one potential splice form that shows a 49 aa substitution for aa 409 - 467, and a second splice form that shows a three aa addition to the C-terminus. Over aa 380 - 467, human ZIC3 displays absolute/100% identity to mouse ZIC3.

## Preparation

Sheep antibodies were raised against purified, *E. coli*-derived recombinant human ZIC3 (rhZIC3; aa 380 - 467; Accession # O60481). 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% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.

## 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 ZIC3 in Western blot.

## Application

**Western blot** - An antibody concentration of 1.0 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

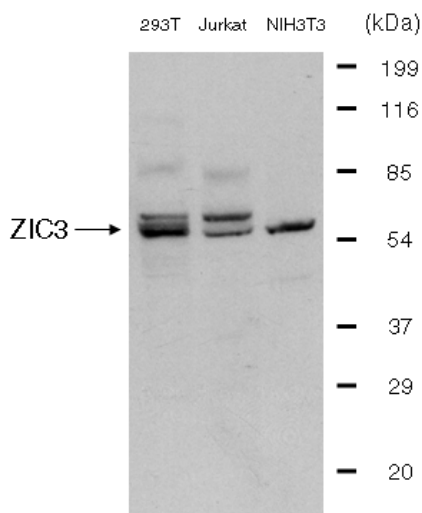
#### Antibody Solution

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 1.0 µg/mL goat anti-human/mouse ZIC3.
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.**



## Detection of ZIC3 with AF5310.

30 µg of extracts from human 293T, Jurkat and mouse NIH3T3 cells were resolved by SDS-PAGE. Following electrophoresis, lysates were transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL anti-ZIC3, as described in *Protocols for Immunoblotting*.