

## ORDERING INFORMATION

**Catalog Number:** AF5720

**Lot Number:** CCTJ01

**Size:** 100 µg

**Storage:** -20° C

**Specificity:** human TACC3

**Immunogen:** *E. coli*-derived recombinant human TACC3 (aa 689 - 838)

**Ig Type:** goat IgG

**Application:** Western blot

## Background

Transforming acidic coiled-coil containing protein 3 (TACC3; also known as ERIC-1) is a 92 - 140 kDa member of the TACC family of transcriptional regulatory proteins. It is expressed in a variety of cell types, including Sertoli cells, endothelial cells, erythroid progenitors, and thyroid epithelium. When phosphorylated by Aurora A on Ser558, it stabilizes the mitotic spindle. It also interacts with nuclear histone acetyltransferases, promoting their positive effect on transcription. Human TACC3 is 838 amino acids (aa) in length and contains one poly-Serine region (aa 155 - 160) and a TACC coiled-coil domain (aa 637 - 837). There is one potential splice variant that shows a Lys substitution for aa 102 - 462. Over amino acids 689 - 838, human TACC3 shares 79% aa identity with mouse TACC3.

## Preparation

Produced in goat immunized with purified, *E. coli*-derived, recombinant human Transforming acidic coiled-coil containing protein 1 (rhTACC3; aa 689 - 838; Accession # Q9Y6A5). Human TACC3 specific IgG was purified by affinity chromatography.

## Formulation

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

## Reconstitution

Reconstitute the antibody in 100 µL PBS containing 0.02% NaN<sub>3</sub>. The antibody concentration will be 1.0 mg/mL.

## 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

The antibody detects endogenous human TACC3 at ~130 kDa by 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

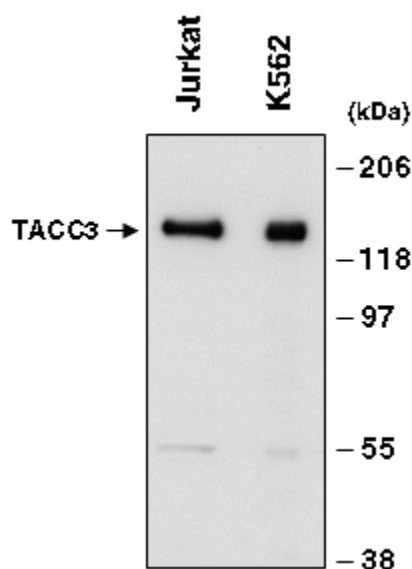
#### Antibody Solution

2% 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 in antibody solution containing 1.0 µg/mL goat anti-human/mouse/rat TACC3.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing a 1:2,000 dilution of HRP-conjugated Donkey anti-goat IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detect with chemiluminescent reagents.

**Cell lysates for Western blottings** - To prepare total cell lysates, cells are solubilized in hot 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at  $2 \times 10^6$  -  $1 \times 10^7$  cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with 3 - 4 bursts of 5 - 10 second each. Samples are diluted with 1X SDS sample buffer to the desired concentration.

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



### Detection of TACC3 with AF5720.

Lysates from human Jurkat and K562 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 1.0 µg/mL goat anti-TACC3 as described in *Protocols for Immunoblotting*.