NEUROMICS

TCF4 (C9B9) Data Sheet

Catalog Number:	RA18033	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human, Mouse, Rat
Immunogen Sequence:	Synthetic peptide (KLH-coupled) corresponding to residues surrounding Glu81 of human TCF4.	**Format:**	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol.
Applications:	Western Blot 1:1000 Immunofluorescence: 1:100 Immunoprecipitation 1:50		
	Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Store at -20ºC. *Do not aliquot the antibody*. Stable for at least 6 months. Repeated freeze/thaw cycles compromise the integrity of the antiserum.		

Application Notes

Description/Data:
LEF1 and TCF are members of the high mobility group (HMG) DNA binding protein family of transcription factors which consists of the following: Lymphoid enhancer factor 1 (LEF1), T Cell Factor 1 (TCF1), TCF3 and TCF4 (1). LEF1 and TCF1 were originally identified as important factors regulating early lymphoid development (2) and act downstream in Wnt signaling. LEF1/TCF bind to Wnt response elements to provide a docking site for β-catenin, which translocates to the nucleus to promote the transcription of target genes upon activation of Wnt signaling (3). LEF1/TCF proteins are dynamically expressed during development and aberrant activation of the Wnt signaling pathway is involved in many types of cancers including colon cancer (4,5). TCF4, also known as TCF7L2, is expressed widely during development. Gene targeting studies indicate that TCF4 is required to maintain the crypt stem cells of the small intestine (6,7). TCF4 has several splicing isoforms that are expressed differentially in tissues and during cancer progression (8,9). Studies also indicate that a variant of the TCF4 gene confers an increased risk of type 2 diabetes (10).

Image: TCF4 (C9B9) staining of HCT-116 cells (left) or SHSY-5Y cells (right) using TCF4 (C9B9) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red).

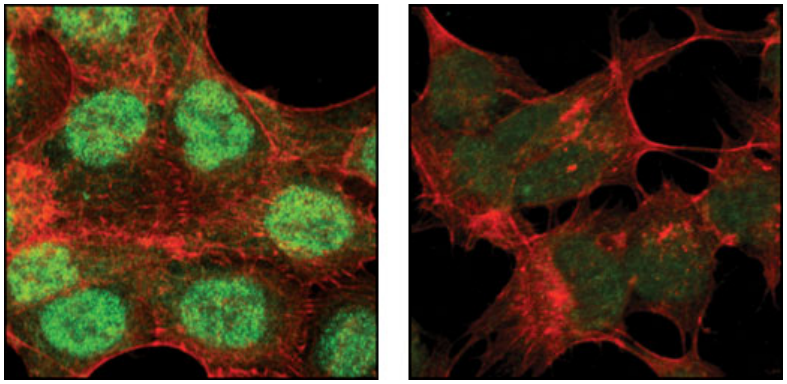

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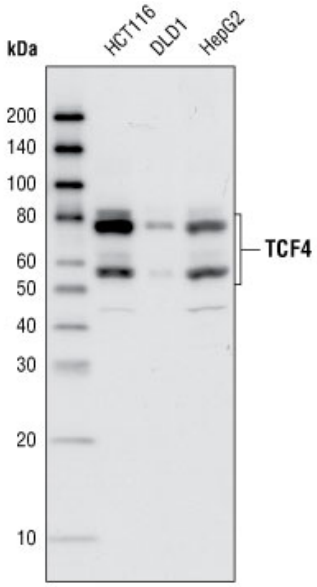
Image: Western blot analysis of total cell lysates from HCT116, DLD1 and HepG2 cells using TCF4 (C9B9) Rabbit mAb.



Specificity:
TCF4 (C9B9) Rabbit mAb detects endogenous levels of total TCF4 protein.

Western Blot:
Sample Preparation
1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape cells from plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose (or PVDF) membrane.
Membrane Blocking and Antibody Incubations
Note: Volumes for 10 cm x 10 cm (100 cm2) membrane; for different sized membranes, adjust vol. accordingly.
1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.
8. Process membranes using enhanced chemiluminescence.

Immunostaining
NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.
1. Block specimen in 5% normal serum from same species as secondary antibody (eg. normal goat serum, normal donkey serum) in PBS/Triton for 60 minutes.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in PBS/Triton. You will need 50-100 µl per section, 25-50 µl per coverslip,

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chamber, or well (48 or 96 well plate).
3. Aspirate blocking solution, apply diluted primary antibody.
NOTE: For double-labeling, prepare a cocktail of mouse and rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 minutes each.
OPTION: To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.
NOTE: If using primary antibodies directly conjugated with AlexaFluor® fluorochromes, then skip to step C8.
6. Incubate in fluorochrome-conjugated secondary antibody diluted in PBS/Triton for 1-2 hours at room temperature in dark.
NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated anti-mouse and anti-rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
7. Rinse in PBS/high salt PBS as in step 5.
8. Coverslip slides with Vectashield Mounting Medium or apply just enough to cover cells in multiwell plate.
9. Seal slides by painting around edges of coverslips with nail polish.
10. Examine specimens immediately using appropriate excitation wavelength, depending on fluorochrome for best results or store flat at 4°C in dark.

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