NEUROMICS

# TCF4 (C48H11) Data Sheet

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| **Catalog Number:** | RA18032 | **Host:** | Rabbit |
| **Product Type:** | Affinity Purified Antibody | **Species Reactivity:** | Human, Mouse, Rat and Chicken |
| **Immunogen Sequence:** | Synthetic peptide (KLH-coupled) corresponding to (KLH-coupled) corresponding to Leu330 of human TCF4. | **Format:** | Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol. |
| **Applications:** | Western Blot 1:1000<br>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)..

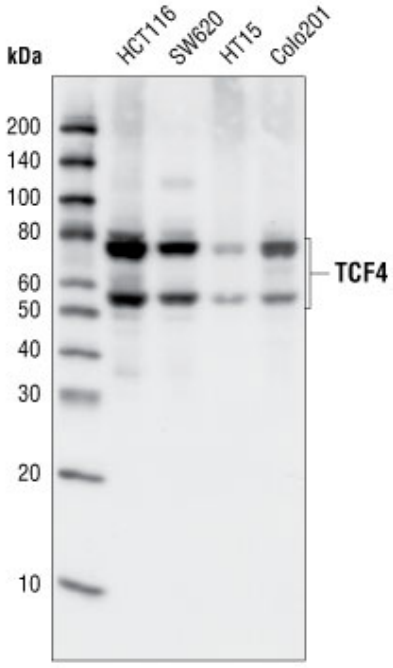


*Image: Western blot analysis of extracts from various cell types using TCF4 (C48H11) Rabbit mAb.*

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**Specificity:**
TCF4 (C48H11) 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.

**Immunoprecipitation:**
Preparing Cell Lysates
1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate the plates on ice for 5 minutes.
4. Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate samples on ice three times for 5 seconds each.
6.Microcentrifuge for 10 minutes at 14,000 X g, 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at –80°C.

**Immunoprecipitation**
Optional: It may be necessary to perform a lysate pre-cleaning step to reduce non-specific binding to the Protein A/G agarose beads (See section below).
1. Take 200 µl cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
2. Add either protein A or G agarose beads (20 µl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500 µl of 1X cell lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.
6. Load the sample (15–30 µl) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

**Cell Lysate Pre-Cleaning (Optional)**
1. Take 200 µl cell lysate and add to either Protein A or G agarose beads (20 µl of 50% bead slurry).
2. Incubate at 4°C for 30 – 60 minutes.

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3. Spin for 10 minutes at 4°C. Transfer the supernatant to a fresh tube.
4. Proceed to step 1 of Immunoprecipitation.

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