

ORDERING INFORMATION

Catalog Number: AF5784

Lot Number: CCSL01

Size: 100 µg

Specificity: human ZBTB38

Immunogen: *E. coli*-derived rhZBTB38
(aa 1 - 232)

Ig Type: goat IgG

Application: Western blot

Background

ZBTB38 (Zinc finger and BTB domain-containing protein 38; also CIBZ and Zenon) is an early member of a family of Kaiso-like DNA binding proteins. Although its predicted MW is 134 kDa, it runs anomalously in SDS-PAGE at 175 kDa. ZBTB38 binds to singly methylated CpG (cytosine-phosphate-guanine) motifs. It is considered a transcriptional repressor that either homodimerizes, or heterodimerizes with ZBTB4, and subsequently interacts with either CtBP-1 or -2 to mediate repression. Human ZBTB38 is 1195 amino acids (aa) in length. It contains a BTB domain (aa 33 - 100), an RD2 region (aa 158 - 341) and ten C2H2-type zinc finger domains (aa 342 - 1147). There is one potential alternate start site at Met595. Over aa 1 - 232, human ZBTB38 shares 96% aa identity with mouse ZBTB38.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human ZBTB38 (rhZBTB38; aa 1 - 232; (Accession # Q8NAP3). 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% Na₂S₂O₃.

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 ZBTB38 in Western blot with an approximate molecular weight of 175 kDa.

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

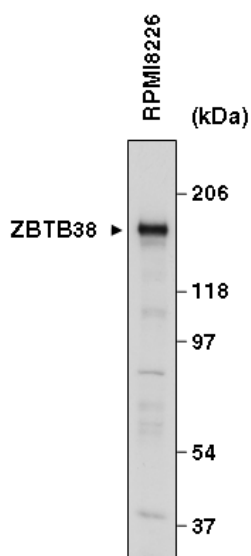
Antibody Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane 1 hour at room temperature in Antibody Solution containing 1.0 µg/mL goat anti-human ZBTB38.
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 1:2000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
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 ZBTB38 with AF5784.

Lysates from human RPMI8226 cells were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with 1.0 µg/mL anti-ZBTB38, as described in *Protocols for Immunoblotting*.