

ORDERING INFORMATION

Catalog Number: AF5780

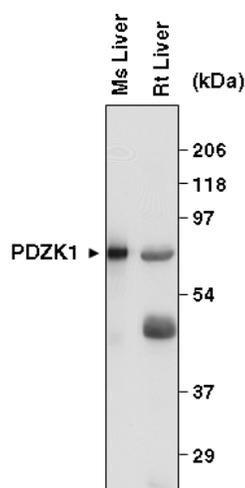
Lot Number: CCSG01

Specificity: mouse and rat PDZK1

Immunogen: *E. coli*-derived rmPDZK1
(aa 1 - 519)

Ig Type: goat IgG

Application: Western blot



Detection of PDZK1 with AF5780.

1 µg of mouse liver and 30 µg rat liver extract resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with 1.0 µg/mL anti-PDZK1, as described in *Protocols for Immunoblotting*.

Background

PDZK1 (PDZ Domain-containing 1; also CFTR-associated protein of 70 kDa/CAP70) is a 65 - 70 kDa member of the NHER (Na/H Exchange Regulator) family of proteins. It is only expressed in epithelium, particularly that found in intestine, kidney and liver. PDZK1 serves as a scaffold protein that connects cell membrane proteins to cytoplasmic regulators. It also serves a critical role in basal-level bicarbonate secretion. Mouse PDZK1 is 519 amino acids (aa) in length and contains four PDZ domains (aa 9 - 90; 128 - 215; 243 - 323; and 378 - 458) plus three phosphorylation sites at Ser489, Ser492 and Ser514. Mouse PDZK1 shares 78% and 91% aa identity with human and rat PDZK1, respectively.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant mouse PDZK1 (rmPDZK1; aa 1 - 519; Accession # Q9JIL4). 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 mouse and rat PDZK1 in Western blot with an approximate molecular weight of 70 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-mouse PDZK1.
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.

Lysates for Western blottings - Tissue extracts are 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.