Anti-Phospho-Thr^{53} NCC Antibody

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Catalog #: p1311-53 **Size:** 100 µl

Cite this Antibody: PhosphoSolutions Cat# p1311-53, RRID:AB_2650477

Host	Applications	Species Tested	Species Reactivity*	Molecular Reference
Rabbit	WB 1:1000 IF 1:100	M	GP, Ha	~160 kDa

Product Description: Affinity purified rabbit polyclonal antibody.

Biological Significance: The thiazide-sensitive sodium chloride cotransporter, NCC, is the major NaCl transport protein in the distal convoluted tubule (DCT) and plays an important role in maintaining blood pressure (Rosenbaek et al., 2014, Feng et al., 2015). Phosphorylation of NCC at Thr^{53}, Thr^{58}, and Ser^{71} is an essential mediator of NCC function (Rosenbaek et al., 2014). NCC is constitutively cycled to the plasma membrane, and upon stimulation, it can be phosphorylated to both increase NCC activity and decrease NCC endocytosis, together increasing NaCl transport in the DCT (Feng et al., 2015).

Antigen: Phosphopeptide corresponding to amino acid residues surrounding the phospho-Thr^{53} of mouse NCC.

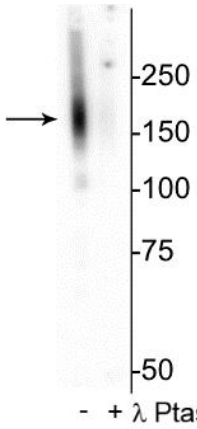
Antibody Specificity: Specific for endogenous levels of the ~160 kDa NCC protein phosphorylated at Thr^{53}. Band of interest smearing likely due to glycosylation. Immunolabeling is completely eliminated by treatment with λ-Ptase.

Purification Method: Prepared from pooled rabbit serum by affinity purification via sequential chromatography on phospho and non-phosphopeptide affinity columns.

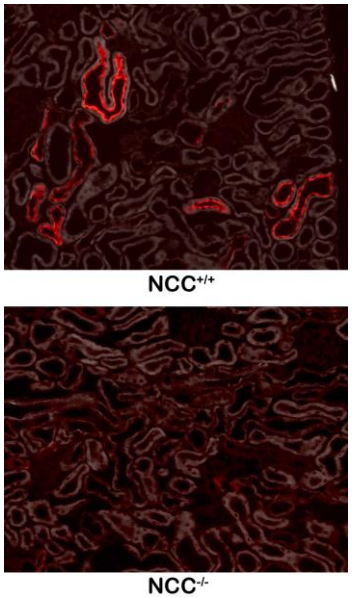
Quality Control Tests: Western blots performed on each lot.

Packaging: 100 µl in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg BSA per ml and 50% glycerol.

Storage and Stability: Shipped on blue ice. Storage at -20°C is recommended, as aliquots may be taken without freeze/thawing due to presence of 50% glycerol. Stable for at least 1 year at -20°C.



Western blot of mouse kidney lysate showing specific immunolabeling of the ~160 kDa NCC protein phosphorylated at Thr^{53} in the first lane (-). Phosphospecificity is shown in the second lane (+) where immunolabeling is completely eliminated by blot treatment with *lambda* phosphatase (λ-Ptase, 1200 units for 30 min).



Immunostaining of PFA perfused frozen kidney sections from WT and NCC KO mice showing specific labeling of the NCC protein phosphorylated at Thr^{53} on the top and the absence of staining in the KO on the left. (Image courtesy of Lauren Miller, Ellison Lab, OHSU.)

Application Key: **WB** = Western Blot **IF** = Immunofluorescence **IHC** = Immunohistochemistry **IP** = Immunoprecipitation
Species Reactivity Key: **All**-All Species **A**-Avian **Amp**-Amphibian **Ar**-*Arabidopsis* **B**-Bovine **C**-Canine **Ch**-Chicken **D**-*Drosophila* **GP**-Guinea Pig **H**-Human **Ha**-Hamster **M**-Mouse **NHP**- Non-human primate **P**-Pig **R**-Rat **S**-Sheep **X**-*Xenopus* **Z**-Zebrafish
*Species assumed based on 100% homology with sequence used as antigen **For Research Use Only**

Product Specific References:

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General References

Feng X, Zhang Y, Shao N, Wang Y, Zhuang Z, Wu P, Lee MJ, Liu Y, Wang X, Zhuang J, Delpire E, Gu D, Cai H. (2015) Aldosterone modulates thiazide-sensitive sodium chloride cotransporter abundance via DUSP6-mediated ERK1/2 signaling pathway. *Am J Physiol Renal Physiol*. 308(10):F1119-27

Rosenbaek LL, Kortenoeven ML, Aroankins TS, Fenton RA. (2014) Phosphorylation decreases ubiquitylation of the thiazide-sensitive cotransporter NCC and subsequent clathrin-mediated endocytosis. *J Biol Chem*. 289(19):13347-61