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### **Product Datasheet**

## Anti-NCC (Thiazide sensitive NaCl cotransporter) (Thr53)

# Pooled Serum KO Validated

Overview

Catalog #	p1311-53
Host Species	Rabbit Polyclonal
Format	Antigen Affinity Purified from Pooled Serum
Applications	WB 1:1000-1:6000 IHC 1:100-1:10,000
Species Tested	Human, Mouse, Rat
Expected Reactivity	Guinea Pig, Hamster
Immunogen	Synthetic phospho-peptide corresponding to amino acid residues surrounding Thr53 of mouse NCC, conjugated to keyhole limpet hemocyanin (KLH).
Molecular Weight	160 kDa
Cite this Antibody	PhosphoSolutions Cat# p1311-53, RRID: AB_2650477

#### Images





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

Immunostaining of PFA perfused frozen kidney sections from WT & NCC KO mice showing specific labeling of the NCC protein phosphorylated at Thr<sup>53</sup> (cat.p1311-53, red, 1:100,000) on the top & the absence of staining in the KO on the bottom. (Image courtesy of Lauren Miller, Ellison Lab, OHSU.)

#### Details

Target Description	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).
Specificity	Specific for endogenous levels of the ~160 kDa NCC protein phosphorylated at Thr53. Band of interest smearing likely due to glycosylation. Immunolabeling is completely eliminated by treatment with $\lambda$ -phosphatase.
Production/Purification	Prepared from pooled rabbit serum by affinity purification via sequential chromatography on phospho and non-phosphopeptide affinity columns.
Quality Control	Western blots performed on each lot.
Buffer	10 mM HEPES (pH 7.5), 150 mM NaCl, 100 $\mu g$ per ml BSA and 50% glycerol.
Storage	Storage at -20°C is recommended, as aliquots may be taken without freeze/thawing due to presence of 50% glycerol.
Stability	After date of receipt, stable for at least 1 year at -20°C.

#### **Significant Citations**

Aly, R., Darwish, S., Bala, N., Ebrahim, A., Shoemaker, L.R., McCray, J., Garrett, T.J. and Alli, A.A. (2024). Functional and metabolomic analysis of urinary extracellular vesicles from juvenile mice with renal compensatory hypertrophy. *Biochimica Et Biophysica Acta. Molecular Basis of Disease*, [online] 1870(5), p.167096.

Nickerson, A.J., Mutchler, S.M., Sheng, S., Cox, N.A., Ray, E.C., Kashlan, O.B., Carattino, M.D., Marciszyn, A.L., Winfrey, A., Gingras, S. and Kirabo, A., 2023. Mice lacking yENaC palmitoylation sites maintain benzamil-sensitive Na+ transport despite reduced ENaC activity. *JCl insight*.

Zietara, A., Palygin, O., Levchenko, V., Dissanayake, L.V., Klemens, C.A., Geurts, A., Denton, J.S. and Staruschenko, A., 2023. Kir7. 1 knockdown and inhibition alter renal electrolyte handling but not the development of hypertension in Dahl salt-sensitive rats. *American Journal of Physiology-Renal Physiology*, 325(2), pp.F177-F187.

Cai, L., Wang, D., Gui, T., Wang, X., Zhao, L., Boron, W.F., Chen, L.M. and Liu, Y., 2023. Dietary sodium enhances the expression of SLC4 family transporters, IRBIT, L-IRBIT, and PP1 in rat kidney: Insights into the molecular mechanism for renal sodium handling. *Frontiers in Physiology*, 14, p.554.

Gao, Z.X., Wei, Q.C., Shu, T.T., Li, S.T., Zhou, R., Li, M.Y., Mao, Z.H., Liu, D.W., Liu, Z.S. and Wu, P., 2023. Kir4. 1 deletion prevents salt-sensitive hypertension in early streptozotocin-induced diabetic mice via Na+–Cl– cotransporter in the distal convoluted tubule. *Journal of Hypertension*, 41(6), pp.958-970.

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