PRODUCT INFORMATION

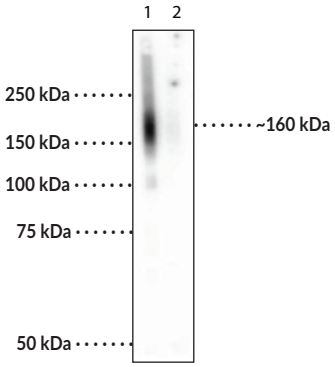
NCC (Phospho-Thr^{53}) Polyclonal Antibody

Item No. 29285

Overview and Properties

Contents:	This vial contains 100 µl of affinity-purified rabbit polyclonal antibody.
Synonym:	Sodium Chloride Symporter
Immunogen:	Phosphopeptide corresponding to amino acid residues surrounding the phospho-Thr^{53} of mouse NCC
Molecular Weight:	~160 kDa
Cross Reactivity:	(+) NCC (phospho-Thr^{53}); (-) Unphosphorylated NCC
Species Reactivity:	(+) Human, mouse, rat
Form:	Liquid
Storage:	-20°C (as supplied)
Stability:	≥1 year
Storage Buffer:	10 mM HEPES, pH 7.5, with 150 mM sodium chloride, 100 µg BSA per ml, and 50% glycerol
Host:	Rabbit
Applications:	Immunofluorescence (IF) and Western blot (WB); the recommended starting dilution is 1:100-1:10,000 and 1:1,000-1:6,000, respectively. Other applications were not tested, therefore optimal working concentration/dilution should be determined empirically.

Image



Lane 1: NCC protein phosphorylated at Thr^{53}
Lane 2: Phosphospecificity

WB 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, 1,200 units for 30 min).

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Description

The thiazide-sensitive sodium chloride cotransporter (NCC) is a member of the SLC12 family of transporters and is encoded by *SLC12A3* in humans.[1] NCC is expressed in epithelial cells of the distal convoluted tubule and localizes to the apical plasma membrane where it mediates sodium reabsorption in the kidney. It consists of a central hydrophobic domain with 12 transmembrane helices that contain affinity-modifying residues for sodium, chloride, and thiazides that is flanked by intracellular N- and C-terminal domains with sites that are subject to phosphorylation. Phosphorylation of NCC at threonine 53 (Thr^{53}) is mediated by serum- and glucocorticoid-inducible kinase 1 (SGK1), STE20/SPS1-related proline-alanine-rich protein kinase (SPAK), or oxidative stress-responsive kinase 1 (OSR1). *X. laevis* oocytes expressing a threonine-to-alanine substitution at Thr^{53} in NCC, which abolishes its phosphorylation, have reduced chloride deprivation-induced sodium uptake, but not NCC surface expression, compared to wild-type oocytes.[2] NCC (phospho-Thr^{53}) levels are increased in the kidney by low dietary intake of sodium chloride or potassium in mice.[3] NCC (phospho-Thr^{53}) levels are also increased in the kidney of the CUL3-Het/Δ9 mouse model of familialhyperkalemic hypertension.[4] Cayman's NCC (Phospho-Thr^{53}) Polyclonal Antibody can be used for immunofluorescence (IF) and Western blot (WB) applications. The antibody recognizes NCC (Phospho-Thr^{53}) at approximately 160 kDa from human, mouse, and rat samples.

References

1. Moes, A.D., van der Lubbe, N., Zietse, R., *et al.* The sodium chloride cotransporter SLC12A3: New roles in sodium, potassium, and blood pressure regulation. *Pflugers Arch.* **466(1)**, 107-118 (2014).
2. Pacheco-Alvarez, D., Cristóbal, P.S., Meade, P., *et al.* The Na^+:Cl^- cotransporter is activated and phosphorylated at the amino-terminal domain upon intracellular chloride depletion. *J. Biol. Chem.* **281(39)**, 28755-28763 (2006).
3. Vallon, V., Schroth, J., Lang, F., *et al.* Expression and phosphorylation of the Na^+-Cl^- cotransporter NCC in vivo is regulated by dietary salt, potassium, and SGK1. *Am. J. Physiol. Renal Physiol.* **297(3)**, F704-F712 (2009).
4. Ferdaus, M.Z., Miller, L.N., Agbor, L.N., *et al.* Mutant cullin 3 causes familial hyperkalemic hypertension via dominant effects. *JCI Insight* **2(24)**, e96700 (2017).

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