

100 µl
(10 western blots)

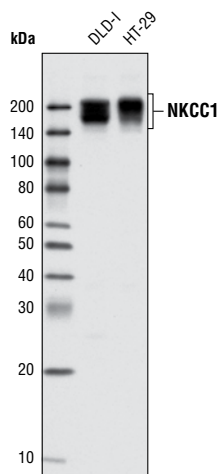
New 10/09

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP Endogenous	H	160-200 kDa	Rabbit**

Background: The electroneutral cation-chloride-coupled co-transporter (SLC12) gene family is comprised of bumetanide-sensitive Na⁺/K⁺/Cl⁻ (NKCC), thiazide-sensitive Na⁺/Cl⁻ and K⁺/Cl⁻ (KCC) co-transporters. SLC12A1/NKCC2 and SLC12A2/NKCC1 regulate cell volume and maintain cellular homeostasis in response to osmotic and oxidative stress (1). The broadly expressed NKCC1 is thought to play roles in fluid secretion (i.e. salivary gland function), salt balance (i.e. maintenance of renin and aldosterone levels) and neuronal development and signaling (2-7). During neuronal development, NKCC1 and KCC2 maintain a fine balance between chloride influx (NKCC1) and efflux (KCC2), which regulates γ -aminobutyric acid (GABA)-mediated neurotransmission (3). Increased NKCC1 expression in immature neurons maintains high intracellular chloride levels that result in inhibitory GABAergic signaling; KCC2 maintains low intracellular chloride levels and excitatory GABAergic responses in mature neurons (4,5,8). Deletion of NKCC1 impairs NGF-mediated neurite outgrowth in PC-12D cells while inhibition of NKCC1 with bumetanide inhibits re-growth of axotomized dorsal root ganglion cells (6,7). Defective chloride homeostasis in neurons is linked to seizure disorders that are ameliorated by bumetanide treatment, indicating that abnormal NKCC1 and NKCC2 expression or signaling may play a role in neonatal and adult seizures (9-12).

NKCC1 is found as a homodimer or within heterooligomers with other SLC12 family members. This transport protein associates with a number of oxidative- and osmotic-responsive kinases that bind, phosphorylate and activate NKCC1 co-transporter activity (13-16). In response to decreased intracellular chloride concentrations, Ste20-related proline-alanine-rich kinase (SPAK) phosphorylates NKCC1 to increase co-transporter activity and promote chloride influx (16-19). Oxidative stress response kinase 1 (OSR1) also phosphorylates and activates NKCC1 in response to oxidative stress (14).



Western blot analysis of whole cell extracts from DLD-1 and HT-29 cells using NKCC1 Antibody.

Specificity/Sensitivity: NKCC1 Antibody recognizes total levels of endogenous NKCC1 protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide (blue carrier-coupled) corresponding to residues surrounding Pro128 of human NKCC1. Antibodies are purified by protein A and peptide affinity chromatography.

Entrez-Gene ID #6558
Swiss-Prot Acc. #P55011

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C.
Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50

For application specific protocols, please see the web page for this product at www.cellsignal.com

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

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- (5) Kahle, K.T. et al. (2008) *Nat Clin Pract Neurol* 4, 490-503.
- (6) Nakajima, K. et al. (2007) *Biochem Biophys Res Commun* 359, 604-10.
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- (8) Ben-Ari, Y. (2002) *Nat Rev Neurosci* 3, 728-39.
- (9) Fukuda, A. (2005) *Nat Med* 11, 1153-4.
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- (12) Kahle, K.T. and Staley, K.J. (2008) *Neurosurg Focus* 25, E22.
- (13) Moore-Hoon, M.L. and Turner, R.J. (2000) *Biochemistry* 39, 3718-24.
- (14) Simard, C.F. et al. (2007) *J Biol Chem* 282, 18083-93.
- (15) Piechotta, K. et al. (2002) *J Biol Chem* 277, 50812-9.
- (16) Dowd, B.F. and Forbush, B. (2003) *J Biol Chem* 278, 27347-53.
- (17) Geng, Y. et al. (2009) *J Biol Chem* 284, 14020-8.
- (18) Smith, L. et al. (2008) *J Biol Chem* 283, 22147-56.
- (19) Gagnon, K.B. et al. (2006) *Mol Cell Biol* 26, 689-98.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.