

**anti-Necdin antibody, rabbit polyclonal (NC243), ChIP grade, KO-Validated**

74-100 100 ug

**Validation of specific reactivity:** Specificity of reaction has been validated with knock-out mice by western blot and IHC-F

**Storage:** Ship at 4°C and store at -20°C. (Do not store below -20°C)

**Reactivity:** React with mouse, rat, human, chicken

**Immunogen:** Recombinant GST-fused mouse necdin (aa 83-325)

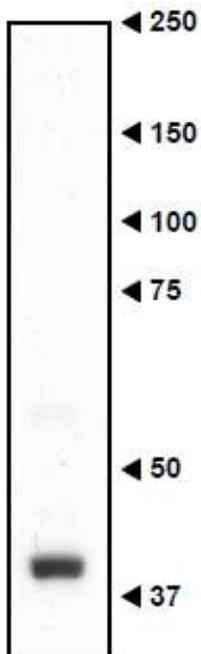
**Applications:**

1. Western blotting (1/1,000-1/3,000)
2. Immunohistochemistry, frozen section (1/500)
3. Immunocytochemistry (1/500)
4. Immunoprecipitation (1/100)
5. Chromatin Immunoprecipitation (1/100)
6. Immunoaffinity assay (Identification of Necdin interacting proteins by column conjugated with anti-Necdin antibody)

**Form:** Protein A affinity purified IgG. 2 mg/ml in PBS, 50% glycerol. Filter-sterilized. No additive.

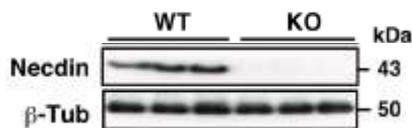
**Background:** **Necdin** (neurally differentiated embryonal carcinoma-derived protein) is a 325-amino acid residue protein encoded by a cDNA clone isolated from neurally differentiated mouse embryonal carcinoma cells (ref.1). **Necdin** is a potent growth suppressor that is expressed predominantly in postmitotic cells such as neurons and muscle cells. **Necdin** has been implicated in the pathogenesis of Prader-Willi syndrome, a human neurodevelopmental disorder associated with genomic imprinting. Furthermore, **necdin** binds to major transcription factors E2F1 and p53, and also to NEFA and nucleobindin, both of which are calcium-binding proteins involved in intracellular calcium homeostasis. From these findings **necdin** is suggested to target various factors involved in the regulation of cell proliferation and survival, and plays a key role in development and differentiation of subsets of neurons in the brain. An antibody (named NC243) against mouse **necdin** was raised in rabbit (ref.1) in the laboratory of Prof. K. Yoshikawa at Osaka Univ.

**Data Link:** Swiss-Prot [P25233](#) (mouse), [Q99608](#) (human)



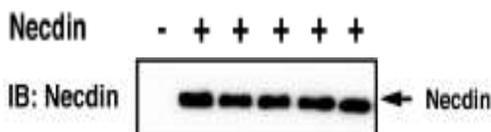
**Fig.1. Western blotting of Necdin in the crude extract of mouse embryo.**

The extract (20  $\mu$ g protein)) was prepared from cerebral cortex of E 16.5 mouse embryo. The anti-Necdin antibody (NC143) was used at 1/3,000 dilution. Molecular mass of mouse Necdin is 37 kDa. The larger size reported here and elsewhere (see Ref) may reflect post-translational modifications such as ubiquitination and phosphorylation at several sites (Swiss-Prot)



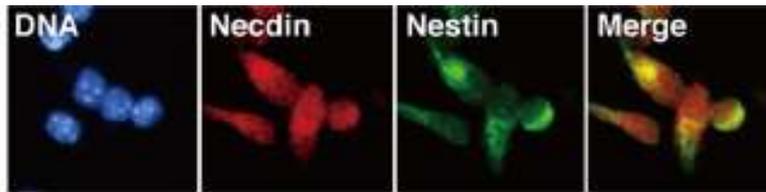
**Fig.2 Validation of the anti-necdin antibody with knock-out mice.**

Proteins in forebrain lysates from wild-type and necdin knock-out mouse embryos at E14.5 were analyzed by Western blotting. Each lane represents the extract from one littermate. Protein levels were normalized to  $\beta$ -tubulin. (Image from Minamido R et al. *PLoS One*. 9 (1) PMID: [24392139](https://pubmed.ncbi.nlm.nih.gov/24392139/).)

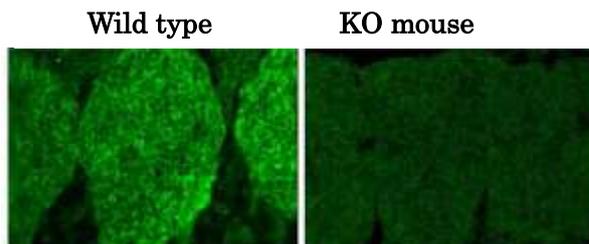


**Fig.3 Immunoprecipitation of necdin**

HEK293A cells were transfected with expression vectors for necdin (+). Cell lysates were immunoprecipitated and immunoblotted with anti-necdin antibody. HEK293A cell lysate (-) is a negative control. (Image from Minamido R et al. *PLoS One*. 9 (1) PMID: [24392139](https://pubmed.ncbi.nlm.nih.gov/24392139/).)

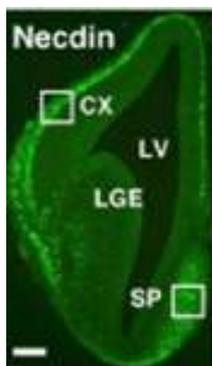


**Fig 4. Immunofluorescence staining of necdin.** Expression of necdin, and nestin in primary neural precursor cells (NPCs) from mouse neocortex. Primary NPCs were prepared from the neocortex at E14.5 and subjected to double-immunostaining for necdin and nestin. DNA was stained with Hoechst 33342. Necdin was immunostained with anti-necdin antibody (NC243) and Nestin with anti-nestin antibody (ST1; BioAcademia 73-105) (Images from Minamido R et al. *PLoS One*. **9** (1) PMID: [24392139](https://pubmed.ncbi.nlm.nih.gov/24392139/).)



**Fig.5 Immunohistochemistry of necdin : Validation of anti-necdin antibody (NC243) with KO-mouse.**

Cryosections of cervical dorsal root ganglion tissues from wild-type (WT) and necdin-null (KO) mice at E14.5 were prepared and immunostained for necdin. Antibody was used at 1/500 dilution



**Fig.6 Immunohistochemical staining of necdin in mouse forebrain.**

E13.5 forebrain cryosections were immunostained for necdin. CX, Cortex; LV, lateral ventricle; LGE, lateral ganglionic eminence; SP, septum. The antibody was used at 1/500 dilution.

**References:** This antibody has been described in ref.1 and used in ref.1-14

1. Niinobe M *et al.* Cellular and subcellular localization of necdin in fetal and adult mouse brain. *Dev Neurosci* (2000) **22**: 310-319 PMID: [10965153](#). **WB, IHC-F (mouse)**
2. Taniguchi N. et al The Postmitotic Growth Suppressor Necdin Interacts with a Calcium-binding Protein (NEFA) in Neuronal Cytoplasm. *J Biol Chem.* 2000 Oct 13;275(41):31674-81. PMID:[10915798](#). **WB, IP, IHC-F, Immunoaffinity column (mouse)**
3. Kobayashi M. et al. Ectopic Expression of Necdin Induces Differentiation of Mouse Neuroblastoma Cells. *J Biol Chem.* 2002 Nov 1;277(44):42128-35. PMID:[12198120](#) **WB, IP (mouse)**
4. Tcherpakov M. et al. The p75 Neurotrophin Receptor Interacts with Multiple MAGE Proteins. *J Biol Chem.* 2002 Dec 20;277(51):49101-4. PMID:[12414813](#) **WB, IF (rat)**
5. Andrieu A. et al. Expression of the Prader-Willi gene Necdin during mouse nervous system development correlates with neuronal differentiation and p75NTR expression. *Gene Expr Patterns.* 2003 Dec;3(6):761-5. PMID:[14643685](#) **IHC (mouse), KO Validation**
6. Kuwajima T *et al* Necdin interacts with the Msx2 homeodomain protein via MAGE-D1 to promote myogenic differentiation of C2C12 cells. *J Biol Chem* (2004) **279**: 40484-40493 PMID: [15272023](#). **WB, IP, IF, IHC (mouse)**
7. Brunelli S. et al. Msx2 and necdin combined activities are required for smooth muscle differentiation in mesoangioblast stem cells. *Circ Res.* 2004 Jun 25;94(12):1571-8. PMID:[15155529](#). **IHC-F (mouse)**
8. Hoek K. et al. Expression Profiling Reveals Novel Pathways in the Transformation of Melanocytes to Melanomas. *Cancer Res.* 2004 Aug 1;64(15):5270-82. PMID:[15289333](#). **WB, IF (human)**
9. Kuwako K. et al Disruption of the Paternal Necdin Gene Diminishes TrkA Signaling for Sensory Neuron Survival. *J Neurosci.* 2005 Jul 27;25(30):7090-9. PMID:[16049186](#) **WB,IP, IHC-F, Immunoaffinity assay, (mouse), KO-Validation for WB and IHC-F**
10. Goldfine AB. et al. Necdin and E2F4 Are Modulated by Rosiglitazone Therapy in Diabetic Human Adipose and Muscle Tissue. *Diabetes.* 2006 Mar;55(3):640-50. PMID:[16505226](#). **WB, IF, (mouse)**
11. Kuwajima T et al. Necdin promotes GABAergic neuron differentiation in cooperation with Dlx homeodomain proteins. *J Neurosci.* (2006) **26**(20):5383-92. PMID:[16707790](#) **WB, IHC-F, IP, Immunoaffinity assay, (mouse)**
12. Andrieu D. et al. Sensory defects in Necdin deficient mice result from a loss of sensory neurons correlated within an increase of developmental programmed cell death. *BMC Dev Biol.* 2006 Nov 20;6:56. PMID: [17116257](#). **WB (mouse)**
13. López-Sánchez N. et al. Single *mage* gene in the chicken genome encodes CMage, a

- protein with functional similarities to mammalian type II Mage proteins. [Physiol Genomics](#). 2007 Jul 18;30(2):156-71. PMID: [17374844](#) **IF/IC, IHC-F (mouse)**
14. Kurita M *et al* Necdin downregulates Cdc2 expression to attenuate neuronal apoptosis. *J Neurosci* (2006)**26**: 12003-12013 PMID: [17108174](#). **WB, ChIP, IF, IHC-F (mouse), KO-Validation for WB**
15. Kubota Y. et al. Necdin restricts proliferation of hematopoietic stem cells during hematopoietic regeneration. [Blood](#). 2009 Nov 12;114(20):4383-92. PMID:[19770359](#) **IF (mouse)**
16. Morillo SM. et al. Nerve Growth Factor-Induced Cell Cycle Reentry in Newborn Neurons Is Triggered by p38MAPK-Dependent E2F4 Phosphorylation. [Mol Cell Biol](#). 2012 Jul;32(14):2722-37. PMID:[22586272](#). **WB, IP, (chicken)**
17. Minamide R *et al* Antagonistic Interplay between Necdin and Bmi1 Controls Proliferation of Neural Precursor Cells in the Embryonic Mouse Neocortex. *PLoS One*. (2014) **9** (1) PMID: [24392139](#). **WB, IF, IP (mouse). Validated with KO mice for WB and IHC-F**
18. Fujimoto I. et al. Necdin controls EGFR signaling linked to astrocyte differentiation in primary cortical progenitor cells. [Cell Signal](#). 2016 Feb;28(2):94-107. PMID: [26655377](#) **WB, IP, (mouse)**
19. Hasegawa K. et al. Promotion of mitochondrial biogenesis by necdin protects neurons against mitochondrial insults. [Nat Commun](#). 2016 Mar 14;7:10943. PMID: [26971449](#). **WB, IHC-F, (mouse) Validated with KO mice for WB**