



anti-Necdin antibody, rabbit serum (NC243)

74-100 100 µl

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.2).

Applications:

1. Western blotting (1,000~3,000 fold dilution) 2. Immunohistochemistry (500~1,000 fold dilution)

3. Immunocytochemistry (500~1,000 fold dilution)

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

Specificity: React with mouse, rat and human Necdin.

Form: Undiluted antiserum added with 0.05% sodium azide.

Storage: Shipped and stored at 4 for a few months. For longer period, aliquot and store at -20

Data Link: Swiss-Prot P25233 (mouse), Q99608 (human)

References: This antibody was produced in ref.2 and used in ref.2, 3 and 4.

 Maruyama K et al (1991) "A novel brain-specific mRNA encoding nuclear protein (necdin) expressed in neurally differentiated embryonal carcinoma cells." Biochem Biophys Res Commun 178: 291-296 PMID: 2069569

- 2. Niinobe M *et al.* (2000) "Cellular and subcellular localization of necdin in fetal and adult mouse brain." *Dev Neurosci* **22**: 310-319 PMID: <u>10965153</u>
- 3. Kuwajima T *et al* (2004) "Necdin interacts with the Msx2 homeodomain protein via MAGE-D1 to promote myogenic differentiation of C2C12 cells." *J Biol Chem* **279**: 40484-40493 PMID: <u>15272023</u>
- Kurita M et al (2006) "Necdin downregulates Cdc2 expression to attenuate neuronal apoptosis." J Neurosci 26: 12003-12013 PMID: 17108174

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to be continued

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Fig.1 Western blotting using this antibody.

Western blot analysis of necdin and MAGE-D1 in mouse embryonal tissues (ref.3).

Tissue lysates (20 ug) from E18.5 embryos were separated by SDS-PAGE and immunoblotted with antibodies against necdin, MAGE-D1 (D1) and tubulin.

Endogenous ~43-kDa necdin protein was detected almost exclusively in the brain and skeletal muscle whereas endogenous ~85-kDa MAGE-D1 protein was expressed in a ubiquitous manner.

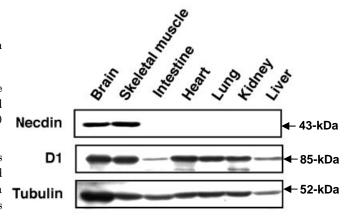


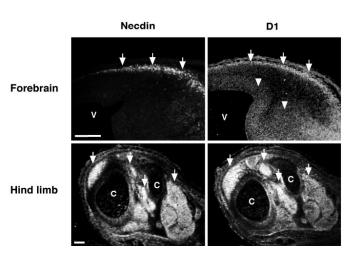
Fig.2 Immunohistochemistry using this antibody (ref.3).

Fluorescence immunohistochemistry for necdin and MAGE-D1 in mouse embryos.

Adjacent sections of the forebrain at E12.5 embryo (upper panels) and the hind limb at E14.5 (lower panels) were stained with antibodies against necdin and MAGE-D1.

The arrows point to the preplate (upper panels) and the skeletal muscles (lower panels). The arrowheads indicate the ventricular proliferative zone; V, ventricle; C, bone cavity.

Necdin and MAGE-D1 were concentrated in the preplate of the forebrain at E12.5 and skeletal muscle tissues in the hind limb at E14.5. In developing neural tube, MAGE-D1 immunoreactivity was distributed in the ventricular zone as well as the marginal zone.



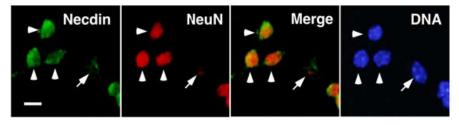


Fig.3 Immunocytochemistry using this antibody (ref.4).

Primary cerebellar granule neurons (CGNs) were fixed and double stained for necdin and postmitotic neuron marker, NeuN. Chromosomal DNA was stained with Hoechst 33342. Arrowheads point to CGNs, and arrows point to CGN progenitors or non-neuronal cells.

Necdin-immunoreactive cells overlapped with NeuN-immunoreactive cells. Necdin immunoreactivity was distributed in both the nucleus and the cytosol.

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