Anti-MAGE-D1 / Dlxin-1 / NRAGE antibody, rabbit polyclonal (MD1)

Product code	74-707
Size	100 µg
Storage	-20℃
Concentration	1.0 mg/ml
Buffer	PBS- with 50% glycerol
Purity	Purified IgG fraction with protein A from rabbit antiserum (74-112).
Immunogen	Recombinant MBT-fused mouse MAGE-D1 (aa 1-775).
Isotype	Rabbit IgG
Reactivity	Mouse, rat and human MAGE-D1
Special notes	Purified from rabbit antiserum (74-112)
Application	1. Western blotting (dilution: 1/3,000-1/1,000) 2. Immunohistochemistry (dilution: 1/300) 3. Immunocytochemistry (dilution: 1/1,000-1/500) 4. ELISA　Not tested for other applications
Background	**MAGE-D1** (**melanoma-associated antigen D1**), also known as **Dlxin-1** or **NRAGE,** is a member of the MAGE protein family. **MAGE-D1** is expressed in almost all normal adult tissues and has been demonstrated to interact with the p75 neutrophin receptor and to facilitate nerve growth factor-dependent apoptosis (ref.1). **MAGE-D1** also interacts with its homologous protein, necdin, which is known as a growth suppressor and a promoter of differentiation in neurons. Necdin binds to Msx (msh homeobox) and Dlx (distal-less homeobox) family homeodomain transcription factors via **MAGE-D1.** These proteins cooperate to modulate differentiation of neurons and muscle cells by regulating gene expression whereas Msx proteins function as transcriptional repressor and Dlx proteins function as transcriptional activators (ref.2 and 3). An antibody (named MD1) against mouse **MAGE-D1** was raised in rabbit (ref.2).
Data Link	UniProtKB　Q9QYH6 **(mouse),** Q9ES73 **(rat),** Q9Y5V3 **(human)**
Related Products	74-100 anti-Necdin antibody. 74-112 anti-MAGE-D1 / Dlxin-1 / NRAGE antibody, rabbit serum (MD1) 74-114 anti-MAGE-G1 / Necdin-like 2 antibody
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.	

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Data Images: 74-707 anti-MAGE-D1 / Dlxin-1 / NRAGE antibody, rabbit polyclonal (MD1)

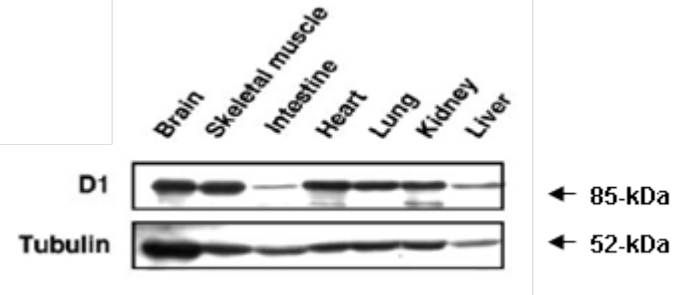


Fig.1 Western blot analysis of MAGE-D1 expression in various organs in mouse embrio.

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

Endogenous ~85-kDa MAGE-D1 protein was expressed in a ubiquitous manner.
Tubulin was used as internal control.

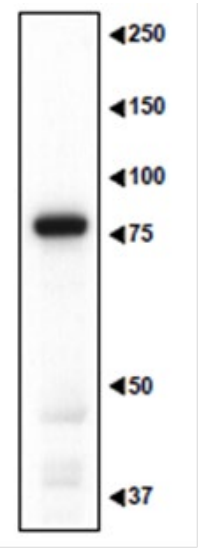


Fig.2.Western blotting of MAGE-D1 in the crude extract of mouse embryo.

The extract (10μg protein) was prepared from cerebral cortex of E 16.5 mouse embryo. The anti-MAGE-D1 antibody (MD1) was used at 1/3,000 dilution. Molecular mass of mouse MAGE-D1is 86 kDa. The numbers are positions of marker proteins shown in kDa.

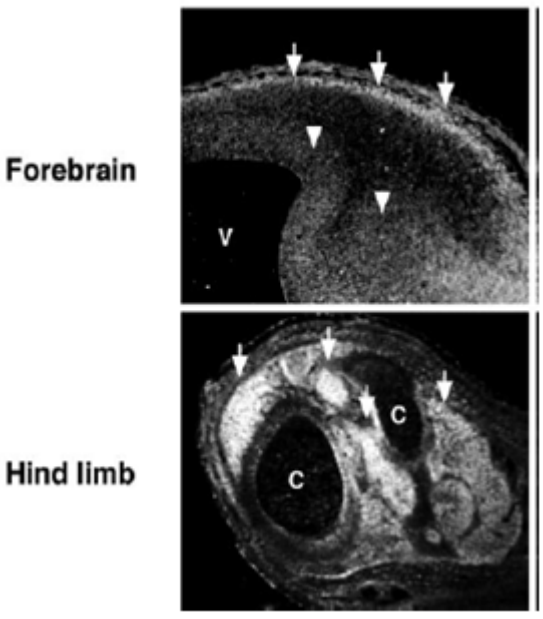


Fig.3 Immunohistochemistry for MAGE-D1 in mouse embryos.

Cryostat section of the paraformaldehyde fixed forebrain at E12.5 embryo (upper panel) and the hind limb at E14.5 (lower panel) were stained with antibody against MAGE-D1 at 1/300 dilution.

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

MAGE-D1 was 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.

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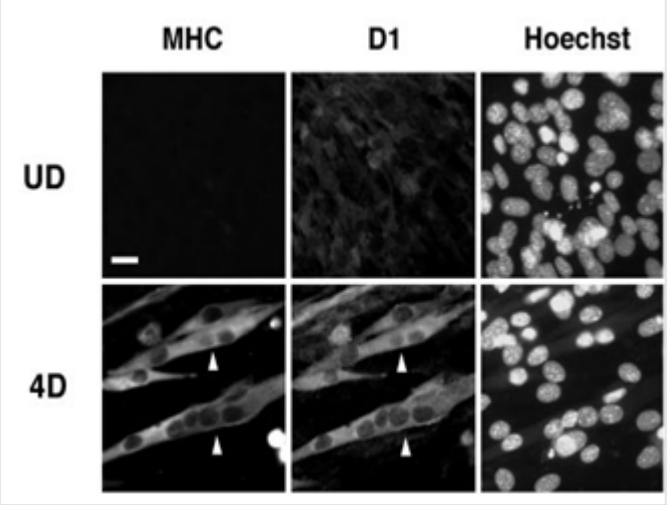


Fig.4 Immunocytochemistry using this antibody .

C2C12 myoblast cells were cultured under undifferentiated conditions (UD) or under differentiation conditions for 4 days (4D). Cells were fixed and triply stained for myosin heavy chain (MHC) with anti-MHC antibody (non BioAcademia product) and for MAGE-D1 (D1) with anti-MAGE-D1 antibody, and for nuclear DNA with Hoechst33342 (Hoechst). The arrowheads point to differentiated multinucleated myocytes.

MHC and MAGE-D1 were distributed predominantly in the cytosol of multinucleated differentiated C2C12 cells.

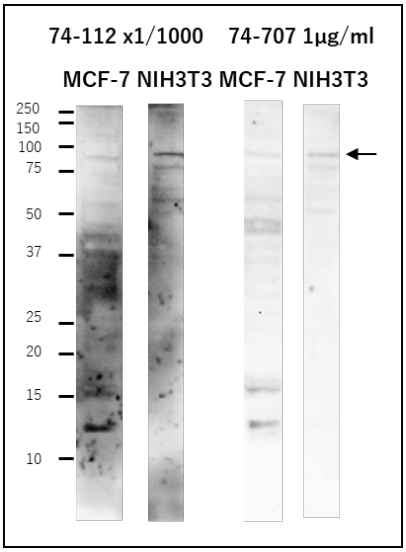


Fig.5.Western blotting of MAGE-D1 in the crude extract.

The extract (MCF-7, NIH3T3 cell 20μg) were applied. The anti-MAGE-D1 antibody (MD1) was used at 1/1000 dilution. Molecular mass of mouse MAGE-D1is 86 kDa. The numbers are positions of marker proteins shown in kDa.

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

1. Salehi AH *et al* (2000) "NRAGE, a novel MAGE protein, interacts with the p75 neutrophin receptor and facilitates nerve growth factor dependent apoptosis." *Neuron* **27**: 279-288 PMID: 10985348
2. 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: 15272023
3. Kuwajima T *et al* (2006) "Necdin promotes GABAergic neuron differentiation in cooperation with Dlx homeodomain proteins." *J Neurosci* **26**: 5383-5392 PMID: 16707790

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