

PRODUCT DATA SHEET

Revised: 05 November 2004
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Product name(s):	Rabbit polyclonal antiserum to human dishevelled-2 (hdvl-2)				
Catalogue number:	DA 4470	Batch number:	Z03389	Expiry date:	12 months from receipt

Introduction:

The *Drosophila* segment polarity gene dishevelled (dsh) encodes a cytoplasmic protein which has been shown to be the convergence point between the wingless (Wg) and Notch signalling pathways¹. Homologous genes have been identified in mammals, and three isoforms of dishevelled (dvl-1, -2 and -3) are now known to exist in humans²⁻⁴ and mice⁵⁻⁸. A rat dvl-1 homologue has recently been described⁹. Each isoform contains distinct domains – N-terminal DIX (domain present in dishevelled and axin, also known as DAX), PDZ and DEP (domain present in dishevelled, Egl-10 and pleckstrin). The DIX domain is important for protein-protein interactions and for the regulation of β -catenin stability^{10,11}, whereas the DEP domain transduces signals to effector proteins downstream of dvl in the Wnt (mammalian Wg homologue) pathway¹²⁻¹³. Recently, all three human dishevelled isoforms have been shown to regulate α -secretase cleavage of amyloid precursor protein (APP), via JNK, PKC and MAP-kinase signalling¹⁴. As dvl-1 inhibits GSK-3 β activity¹⁵, both reducing the phosphorylation of tau^{15,16} and regulating microtubule stability¹⁷, dvl may be of particular importance in the maintenance of neuronal structure and function.

Product information:

The antiserum was raised in a rabbit (94/2) to a synthetic peptide (H-**RGGEASGTSDDGGPC**-NH₂) corresponding to amino acid residues 633-645(+Cys.NH₂) of the human dishevelled 2 protein³ and conjugated to keyhole limpet haemocyanin via the C-terminal cysteine residue using MBS. The sequence exhibits three amino acid substitutions in the aligned sequence of mouse dvl-2 (A⁶³⁷→P; S⁶³⁸→G; S⁶⁴¹→G)^{5,9}. The amino acid sequence does not exist in human dvl-2 or dvl-3.

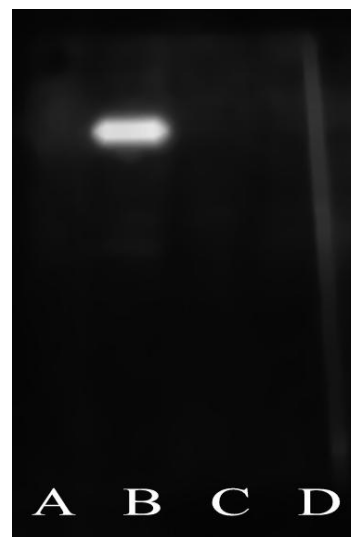
The antiserum has been partially purified by caprylic acid and ammonium sulphate precipitation.

Application data

The antiserum has been characterised by Western blotting against recombinant human dvl-1, -2 and -3 proteins (see Figure). The immunostaining is fully abolished by pre-adsorption of the antibody with cognate peptide (DP 9447).

The antibody may be used in Western blotting applications at dilutions of 1:1000*.

NOTES: *Optimal dilutions must be determined by experimentation, and when used in combination with sensitive detection methods, such as enhanced chemiluminescence (ECL; Nycomed Amersham).



Lane A – HEK293/dvl-1 transfected lysate; lanes B & D – HEK293/dvl-2; lane C HEK293/dvl-3. Lanes A-C antiserum DA4470 used at 1:1000; lane D - as for lanes A-C but pre-adsorbed with cognate peptide. Overnight incubation at 4°C with ECL detection (1 min).

Vial contents, Storage and Use:

Vial contains an immunoglobulin preparation suspended in phosphate-buffered saline (pH 7.4) containing 0.01M sodium azide.

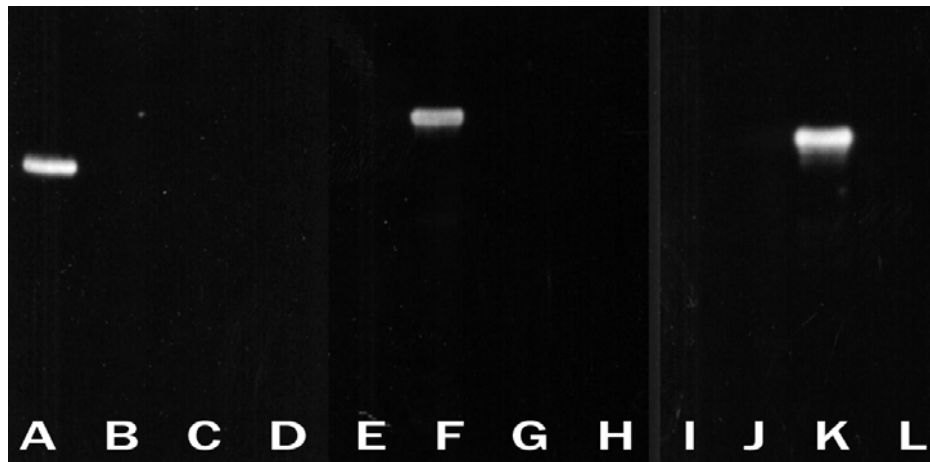
Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes (not less than 10 μ L) prior to freezing if appropriate. The use of high quality 'antiserum-grade' plastic or glass vials is recommended. Store diluted antibody at 2-4°C (do not freeze) and use within 1 month.

Dilute to working strength with 50mM PBS (pH 7.4) containing 1.5% sodium chloride and 1% normal goat serum (if a goat anti-rabbit IgG linker antibody is to be used).

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Comparative results:



Composite Western blot image of antisera DA4170 (dvl-1), DA4270 (dvl-2) and DA4370 (dvl-3) on transfected HEK293 cell lysates. Lanes A, E & I – 6 μ L HEK293/dvl-1 transfected cell lysate; lanes B, F & J - 6 μ L HEK293/dvl-2 transfected cell lysate; lanes C, G & K - 6 μ L HEK293/dvl-3 transfected cell lysate; lanes D, H & L - 6 μ L HEK293 non-transfected cell (control) lysate. Lanes A-D – rabbit antiserum to dvl-1 (DA4170; 1:500); lanes E-H – rabbit antiserum to dvl-2 (DA4270; 1:5000); lanes I-L – rabbit antiserum to dvl-3 (DA4370; 1:5000). Indirect immunoperoxidase detection using ECL (1min exposure).

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