

# PRODUCT DATA SHEET

Revised: 15 November 2004  
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Product name(s):	Rabbit polyclonal antiserum to dishevelled-3 (dvl-3)		
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Catalogue number:	DA 4370	Batch number:	Z01979	Expiry date:	12 months from receipt
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## 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 pathways<sup>1</sup>. Homologous genes have been identified in mammals, and three isoforms of dishevelled (dvl-1, -2 and -3) are now known to exist in humans<sup>2-4</sup> and mice<sup>5-8</sup>. A rat dvl-1 homologue has recently been described<sup>9</sup>. 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  $\beta$ -catenin stability<sup>10,11</sup>, whereas the DEP domain transduces signals to effector proteins downstream of dvl in the Wnt (mammalian Wg homologue) pathway<sup>12-13</sup>. Recently, all three human dishevelled isoforms have been shown to regulate  $\alpha$ -secretase cleavage of amyloid precursor protein (APP), via JNK, PKC and MAP-kinase signalling<sup>14</sup>. As dvl-1 inhibits GSK-3 $\beta$  activity<sup>15</sup>, both reducing the phosphorylation of tau<sup>15,16</sup> and regulating microtubule stability<sup>17</sup>, dvl may be of particular importance in the maintenance of neuronal structure and function.

## Product information:

The antiserum was raised in a rabbit (25/2) to a synthetic peptide (H-CGSDRRKEKDPKAGDS-OH) corresponding to amino acid residues (Cys+)582-596 of the human and mouse dishevelled 3 proteins<sup>4,8</sup> and conjugated to keyhole limpet haemocyanin via the N-terminal cysteine residue using MBS. The amino acid sequence does not exist in human or mouse dvl-1 or dvl-2.

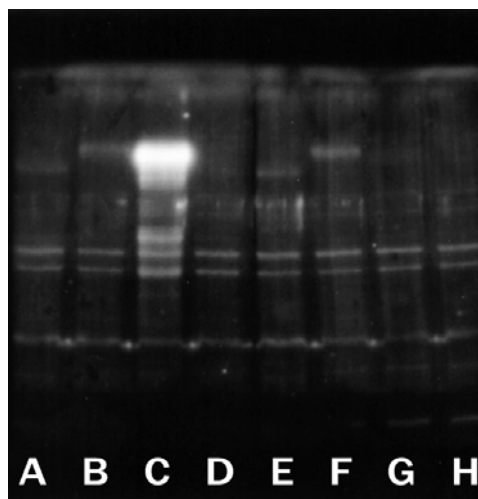
The antiserum has been partially purified by caprylic acid and ammonium sulphate precipitation.

### Application data

The antiserum has been characterised by Western blotting against recombinant human dvl-1, -2 and -3 proteins (see Figure). The immunostaining of the ~90kDa band is fully abolished by pre-adsorption of the antibody with cognate peptide (DP 9437).

The antibody may be used in Western blotting applications at dilutions in excess of 1:1000\*. The weak reactivity observed with dvl-1 and dvl-2 is due to protein-protein interaction, and is not abolished following peptide pre-adsorption.

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



Lanes A&E – HEK293/dvl-1 transfect lysate; lanes B&F – HEK293/dvl-2; lanes C&G HEK293/dvl-3; lanes D&H – HEK293 non-transfected control lysate. Lanes A-D antiserum DA4370 used at 1:1000; lanes E-H as for lanes A-D but pre-adsorbed with cognate peptide. Overnight incubation at 4C 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.

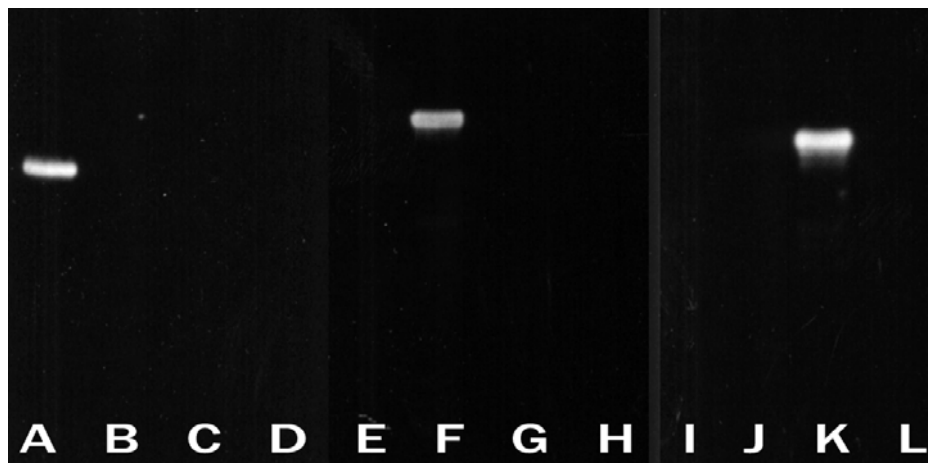
Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes (not less than 10 $\mu$ L) prior to freezing if appropriate. The use of high quality 'antiserum-grade' plastic or glass vials is recommended. Store diluted antibody 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 $\mu$ L HEK293/dvl-1 transfected cell lysate; lanes B, F & J - 6 $\mu$ L HEK293/dvl-2 transfected cell lysate; lanes C, G & K - 6 $\mu$ L HEK293/dvl-3 transfected cell lysate; lanes D, H & L - 6 $\mu$ 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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