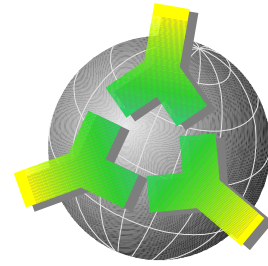


anti-m Diaphanous

rabbit antiserum F1

Lot: A01

data sheet 04/00 — catalog #: 0040-10



immunoGlobe
Antikörpertechnik GmbH

Background information

Drosophila Diaphanous,¹ its mouse homologue p140mDia² (mDia1), mDia2,³ and the human homologues^{4,5} Dia1 and 2 are members of the Formin homology (FH) family of cytoskeletal organizer proteins. Diaphanous proteins contain a Rho-binding domain close to their N-terminus followed by a proline-rich region (designated as FH1 domain) with several poly-proline stretches and a conserved FH2 domain in the middle and C-terminal parts of the protein, respectively.^{2,6,7}

Drosophila Diaphanous is involved in cytokinesis and mutated alleles affect spermatogenesis or oogenesis, respectively, and lead to sterility.¹ The gene encoding a human Diaphanous protein is disrupted in a patient with Premature Ovarian Failure (POF).⁵ Nonsyndromic Deafness DFNA1, an autosomal dominant fully penetrant progressive hearing loss, is associated with a mutation in the human gene for Dia 1.⁴

P140mDia binds profilin and GTP-Rho, and colocalizes with Rho and profilin in membrane lamellae of motile cells and phagocytic cups engulfing fibronectin-coated beads.²

Diaphanous has been suggested to contribute to localized actin polymerization and stress fiber formation, both by recruiting profilin to sites of Rho action and by cooperating with ROCK.^{2,8-10}

P140mDia was also found in association with the cleavage furrow of dividing cells indicating some function during contractile ring formation. Diaphanous proteins are required for cytokinesis, stress fiber formation, and transcriptional activation of the serum response factor (SRF)³.

Antibody preparation and storage

100 µl of crude rabbit antiserum¹¹ F1 containing 0.01% (w/v) Na₂S₂O₃. Vials have been overfilled by 10% to ensure complete recovery of the specified amount. For repeated use store at 4°C (short term), stable for one year from date of shipment when stored at -20°C. Avoid repeated freezing and thawing! Do not store in "frost-free" freezers.

Antigen

The antiserum was raised against an 81 kDa polypeptide (p81)¹¹ that was isolated by profilin affinity chromatography of human platelet lysates and electroeluted after SDS-PAGE. Due to available microsequences comprising two peptides, 25 and 39 amino acids in length¹¹, p81 has been identified as a C-terminal fragment of human Dia1, the homologue of mouse p140mDia². The p81 peptides correspond to human Dia1 amino acids 727-765 (end of FH1 domain) and 1121-1145 (C-terminal coiled coil), respectively.

Species cross-reactivity

human, porcine

Applications

Western (immuno) blotting (1:1000 - 1:1500) with enhanced chemo-luminescence detection. Immunoprecipitation (1:100). All dilution numbers refer to the analysis of human cells and tissues with intermediate to high levels of Dia1 expression and must be viewed as approximate.

Positive control

Human skin fibroblast protein (100 µg), supplied at 1 mg/ml in SDS (Laemmli) sample buffer. Use 25 µl (25 µg) per lane for Western blotting.

Related products

- affinity purified rabbit antibody to lipoma preferred partner (LPP), 50 µg (catalog #0032-05)
- affinity purified rabbit antibody to profilin, 10 µg (catalog # 0022-01)
- monoclonal antibody IE273 to human VASP, 50 µg (catalog # 0016-05)
- rabbit antiserum M4 to human VASP, 100 µl (catalog # 0010-10)
- Pre-immune serum to M4, 25 µl (catalog # 0013-02)
- positive control: human platelet protein in SDS sample buffer, 500 µg (catalog # 8010-50)

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References

(*: papers referring to antiserum F1)

- [1] Castrillon and Wasserman (1994) *diaphanous* is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the *limb deformity* gene. *Development* **120**:3367-3377.
- [2] Watanabe et al. (1997) p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *EMBO J.* **16**:3044-3056.
- [3] Tominaga et al. (2000) Diaphanous-related formins bridge Rho GTPase and Src tyrosine kinase signaling. *Mol. Cell* **5**:13-25.
- [4] Lynch et al. (1997) Nonsyndromic Deafness DFNA1 associated with mutation of a human homolog of the *Drosophila* gene *diaphanous*. *Science* **278**:1315-1318.
- [5] Bione et al. (1998) A human homologue of the *Drosophila melanogaster diaphanous* gene is disrupted in a patient with premature ovarian failure: Evidence for conserved function in oogenesis and implications for human sterility. *Am. J. Hum. Genet.* **62**:533-541.
- [6] Wasserman (1998) FH proteins as cytoskeletal organizers. *Trends Cell Biol.* **8**:111-115.
- [7] Narumiya et al. (1997) Rho effectors and reorganization of actin cytoskeleton. *FEBS Lett.* **410**:68-72.
- [8] Watanabe et al. (1999) Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nature Cell Biol.* **1**:136-143.
- [9] Nakano et al. (1999) Distinct actions and cooperative roles of ROCK and mDia in Rho small G protein-induced reorganization of the actin cytoskeleton in Madin-Darby Canine Kidney cells. *Mol. Biol. Cell* **10**:2481-2491.
- [10] Ridley (1999) Stress fibers take shape. *Nature Cell Biol.* **1**: E64-E66.
- * [11] Reinhard et al. (1995) The proline-rich focal adhesion and microfilament protein VASP is a ligand for profilins. *EMBO J.* **14**:1583-1589.

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