Rabbit (polyclonal) Dab1 [pY^{198}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: 44906G (10 mini-blot size)

Lot Number: See product label

Volume: 100 µL

Form of Antibody: Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

Preservative: 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

Purification: Purified from rabbit serum by Protein A Sepharose affinity chromatography.

Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human Dab1 that contains tyrosine 198. The sequence is conserved in mouse, rat and chicken.

Target Summary: Disabled 1 (Dab1) is an 80 kDa protein that is encoded by the *Disabled-1* gene locus which is mutated in *scrambler* and *yotari* mutant mice. Phenotypically, the mutation of this gene produces motor defects and ataxia, disruption of neuronal migration, and severe cerebellar hypoplasia. Dab1 is an intracellular adapter protein that functions in downstream signaling events initiated by the secreted protein reelin. Dab1 contains a phosphotyrosine binding (PTB) domain in the amino terminus. Tyrosine phosphorylation of Dab1 is increased by reelin binding to the Very Low Density Lipoprotein Receptor (VLDLR) and Apolipoprotein E Receptor 2 (ApoER2) through stimulation of Src family kinases (e.g., Fyn). Src family kinase and c-Abl activities are themselves then stimulated by binding to tyrosine phosphorylated Dab1. Dab1 also mediates activation of Akt (PKB) by reelin resulting in inhibition of glycogen synthase kinase 3β (GSK-3β) and decreased phosphorylation of the microtubule-associated protein, Tau. Dab1 tyrosine 198 is a major site for reelin-induced Src family kinase-mediated phosphorylation in embryonic neurons.

Reactivity: Mouse (100% homologous) Dab1. Human, rat and chicken (100%) Dab1 have not been tested, but are expected to react.

Applications: The antibody has been used in Western blotting. When examining phosphorylation of endogenous Dab1 protein we recommend that Dab1 protein first be immunoprecipitated. Other applications may work but have not been tested.

Suggested Working Dilutions: For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The exact concentration is not determined for each lot; however, the typical range is 0.1-1.0 mg/mL. The optimal antibody concentration should be determined empirically for each specific application.

Storage: Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

Expiration Date: Expires one year from date of receipt when stored as instructed.

Positive Control Used: Human Embryonic Kidney (HEK293) cells transfected with wild-type mouse Dab1 protein.

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Related Products:

Antibodies:

Dab1 [pY^{220}], Cat. # 44908	Akt/PKB [pS^{473}], Cat. # 44623G
Dab1 [pS^{491}], Cat. # 44909G	c-Abl [pY^{245}], Cat. # 44250
Aβ [42], Cat. # 44344	GSK-3α [pY^{279}]/β [pY^{216}], Cat. # 44604G
Aβ [40], Cat. # 44348A	Src Family Kinase Negative Regulatory Site, Cat. # 44912
Akt/PKB [pT^{308}], Cat. # 44602G	Tau Sampler Pack, Cat. # 44779G

References:

Arnaud, L., et al. (2003) Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. Curr. Biol. 13(1):9-17.

Beffert, U., et al. (2002) Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3β. J. Biol. Chem. 277(51):49958-49964.

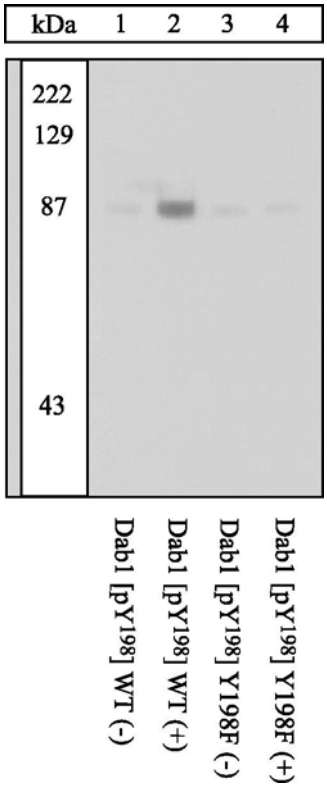
Keshvara, L., et al. (2001) Identification of reelin-induced sites of tyrosyl phosphorylation on disabled 1. J. Biol. Chem. 276(19):16008-16014.

Howell, B.W., et al. (2000) Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. Curr. Biol. 10(15):877-885.

Hiesberger, T., et al. (1999) Direct binding of reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. Neuron 24(2):481-489.

Homayouni, R., et al. (1999) Disabled-1 binds to the cytoplasmic domain of amyloid precursor-like protein 1. J. Neurosci. 19(17):7507-7515.

Howell, B.W., et al. (1999) Reelin-induced tyrosine phosphorylation of disabled 1 during neuronal positioning. Genes Dev. 13(6):643-648.



Up-regulation and Mutant Analysis

Extracts of HEK293 cells transfected with wild type (1, 2) or Y198F mutant mouse Dab1 (3, 4) left untreated (-) (1, 3) or treated with H_2O_2 (+) (2, 4) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer overnight at 4°C then incubated with the Dab1 [pY^{198}] antibody for two hours at room temperature in a 3% BSA-TBST buffer. After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that the antibody does not recognize Dab1 in the Dab1 Y198F mutant-expressing cells, demonstrating the specificity of the antibody. The data also show the induction of Dab1 [pY^{198}] phosphorylation by the addition of H_2O_2 in the wild-type Dab1-expressing cells.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Invitrogen cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween-20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer
Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer
Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline
Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer
Formulation:
100 mL Tris buffered saline
5 gm Ig-free BSA
0.1 mL Tween 20

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