SYSY Antibodies

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Doc2b

Cat.No. 174 103; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For **reconstitution** add 50 µl H_2O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use. Antibodies should be stored at +4°C when still lyophilized. Do not freeze! For detailed information, see back of the data sheet.
Applications	**WB**: 1 : 1000 **IP**: not tested yet **ICC**: not tested yet **IHC**: not tested yet **IHC_P**: not tested yet
Immunogen	Recombinant protein corresponding to AA 38 to 119 from rat Doc2b (UniProt Id: P70610)
Reactivity	Reacts with: rat (P70610), mouse (P70169). Other species not tested yet.
Specificity	Specific for Doc 2b, no cross-reactivity to Doc 2a and Doc 2c. K.O. PubMed: 32347796

TO BE USED IN VITRO / FOR RESEARCH ONLY
NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS

Background

The **Doc 2** protein family comprises the three isoforms Doc 2a, b and c. All three proteins are composed of a short N-terminal Munc 13 binding region followed by two C-terminal calcium binding domains (C2A and C2B). Doc 2a and b are predominantly expressed in brain and colocalize with small secretory vesicles whereas Doc 2c is mainly expressed in the heart.
The exact mechanism how the Doc proteins are involved in Ca^{2+} induced exocytosis has yet to be determined.

Selected References for 174 103

Munc13-1 is a Ca2+-phospholipid-dependent vesicle priming hub that shapes synaptic short-term plasticity and enables sustained neurotransmission.
Lipstein N, Chang S, Lin KH, López-Murcia FJ, Neher E, Taschenberger H, Brose N
Neuron (2021) : . . **WB; tested species: mouse**

Single synapse glutamate imaging reveals multiple levels of release mode regulation in mammalian synapses.
Farsi Z, Walde M, Klementowicz AE, Paraskevopoulou F, Woehler A
iScience (2021) 241: 101909. . **ICC; tested species: rat**

Loss of Doc2b does not influence transmission at Purkinje cell to deep nuclei synapses under physiological conditions.
Khan MM, Regehr WG
eLife (2020) 9: . . **IHC; KO verified; tested species: mouse**

Distinct insulin granule subpopulations implicated in the secretory pathology of diabetes types 1 and 2.
Kreutzberger AJB, Kiessling V, Doyle CA, Schenk N, Upchurch CM, Elmer-Dixon M, Ward AE, Preobraschenski J, Hussein SS, Tomaka W, Seelheim P, et al.
eLife (2020) 9: . . **WB; tested species: rat**

Selected General References

DOC2A and DOC2B are sensors for neuronal activity with unique calcium-dependent and kinetic properties.
Groffen AJ, Friedrich R, Brian EC, Ashery U, Verhage M
Journal of neurochemistry (2006) 973: 818-33. .

Ca(2+)-induced recruitment of the secretory vesicle protein DOC2B to the target membrane.
Groffen AJ, Brian EC, Dudok JJ, Kampmeijer J, Toonen RF, Verhage M
The Journal of biological chemistry (2004) 27922: 23740-7. .

Different spatiotemporal expression of DOC2 genes in the developing rat brain argues for an additional, nonsynaptic role of DOC2B in early development.
Korteweg N, Denekamp FA, Verhage M, Burbach JP
The European journal of neuroscience (2000) 121: 165-71. .

Transient, phorbol ester-induced DOC2-Munc13 interactions in vivo.
Duncan RR, Betz A, Shipston MJ, Brose N, Chow RH
The Journal of biological chemistry (1999) 27439: 27347-50. .

Doc2 is not associated with known regulated exocytotic or endosomal compartments in adrenal chromaffin cells.
Charvin N, Williams G, Burgoyne RD
The Biochemical journal (1999) 341 (Pt 1): 179-83. .

Role of the Doc2 alpha-Munc13-1 interaction in the neurotransmitter release process.
Mochida S, Orita S, Sakaguchi G, Sasaki T, Takai Y
Proceedings of the National Academy of Sciences of the United States of America (1998) 9519: 11418-22. .

Physical and functional interactions of Doc2 and Munc13 in Ca2+-dependent exocytotic machinery.
Orita S, Naito A, Sakaguchi G, Maeda M, Igarashi H, Sasaki T, Takai Y
The Journal of biological chemistry (1997) 27226: 16081-4. .

Access the online factsheet including applicable protocols at https://sysy.com/product/174103 or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All our antibodies and control proteins / peptides are shipped lyophilized (vacuum freeze-dried) and are stable in this form without loss of quality at ambient temperatures for several weeks.

Storage of Sealed Vials after Delivery

- **Unlabeled** and **biotin-labeled antibodies** and **control proteins** should be stored at 4°C before reconstitution. **They must not be stored in the freezer when still lyophilized!** Temperatures below zero may cause loss of performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long term storage (several months) may lead to aggregation.
- **Control peptides** should be kept at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- The storage freezer must not be of the frost-free variety ("no-frost freezer"). This cycle between freezing and thawing (to reduce frost-build-up), which is exactly what should be avoided. For the same reason, antibody vials should be placed in an area of the freezer that has minimal temperature fluctuations, for instance towards the back rather than on a door shelf.
- Aliquot the antibody and store frozen (-20°C to -80°C). Avoid very small aliquots (below 20 µl) and use the smallest storage vial or tube possible. The smaller the aliquot, the more the stock concentration is affected by evaporation and adsorption of the antibody to the surface of the storage vial or tube. Adsorption of the antibody to the surface leads to a substantial loss of activity.
- The addition of glycerol to a final concentration of 50% lowers the freezing point of your stock and keeps your antibody at -20°C in liquid state. This efficiently avoids freeze and thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C.

Monoclonal Antibodies

- **Ascites** and **hybridoma supernatant** should be stored at -20°C up to -80°C. **Prolonged storage at 4°C is not recommended!** Unlike serum, ascites may contain proteases that will degrade the antibodies.
- **Purified IgG** should be stored at -20°C up to -80°C. Adding a carrier protein like BSA will increase long term stability. Many of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Polyclonal Antibodies

- **Crude antisera**: With anti-microbials added, they may be stored at 4°C. However, frozen storage (-20°C up to -80°C) is preferable.
- **Affinity purified antibodies**: Less robust than antisera. Storage at -20°C up to -80°C is recommended. Adding a carrier protein like BSA will increase long term stability. Most of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Fluorescence-labeled Antibodies

- Store as a liquid with 1 : 1 (v/v) glycerol at -20°C. Protect these antibodies from light exposure.

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All our purified antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the amount of deionized water given in the respective datasheet. If higher volumes are preferred, add water as mentioned above and then the desired amount of PBS and a stabilizing carrier protein (e.g. BSA) to a final concentration of 2%. Some of our antibodies already contain albumin. Take this into account when adding more carrier protein.
  For complete reconstitution, carefully remove the lid. After adding water, briefly vortex the solution. You can spin down the liquid by placing the vial into a 50 ml centrifugation tube filled with paper.
- If desired, add small amounts of azide or thimerosal to prevent microbial growth. This is especially recommended if you want to keep an aliquot a 4°C.
- After reconstitution of fluorescence-labeled antibodies, add 1 : 1 (v/v) glycerol to a final concentration of 50%. This lowers the freezing point of your stock and keeps your antibody in liquid state at -20°C.
- Glycerol may also be added to unlabeled primary antibodies. It is a suitable way to avoid freeze-thaw cycles.
- Please refer to our **tips and hints for subsequent storage** of reconstituted antibodies and control peptides and proteins.