SYSY Antibodies

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Otoferlin

Cat.No. 178 003; 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**: not tested yet **IP**: not tested yet **ICC**: not tested yet **IHC**: 1 : 5000 **IHC-P**: not tested yet **iDISCO**: external data (see remarks)
Immunogen	Synthetic peptide corresponding to AA 181 to 196 from mouse Otoferlin (UniProt Id: Q9ESF1-1)
Reactivity	Reacts with: mouse (Q9ESF1). Other species not tested yet. Antigen identical in rat.
Specificity	K.O. validated PubMed: 37248244
Remarks	**iDISCO**: This antibody has been successfully used for iDISCO according to Rankovic et al. 2020.

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

Background

Otoferlin is a transmembrane protein with six C2 domains which locates to synaptic vesicles. It belongs to the family of ferlin C2 proteins which are implicated in membrane-membrane fusion events.
Otoferlin is expressed in hair cells of the inner ear and in some brain regions. Hair cell ribbon synapses lack the candidate neuronal calcium sensors synaptotagmin 1, synaptotagmin 2 and synaptotagmin 3 and other proteins typical for conventional synapses like synaptophysin.
By analogy of Ca^{2+} binding, SNARE-binding and because of some aspects of the physiological phenotype of otoferlin-deficient hair cells, otoferlin is suggested to take the role of a Ca^{2+} sensor of exocytosis in hair cells. Mutations in the otoferlin gene are associated with the recessive prelingual deafness DFNB9.

Selected References for 178 003

Overloaded Adeno-Associated Virus as a Novel Gene Therapeutic Tool for Otoferlin-Related Deafness.
Rankovic V, Vogl C, Dörje NM, Bahader I, Duque-Afonso CJ, Thirumalai A, Weber T, Kusch K, Strenzke N, Moser T
Frontiers in molecular neuroscience (2020) 13: 600051. . **IHC, IDISCO; tested species: mouse**

Cochlear transcript diversity and its role in auditory functions implied by an otoferlin short isoform.
Liu H, Liu H, Wang L, Song L, Jiang G, Lu Q, Yang T, Peng H, Cai R, Zhao X, Zhao T, et al.
Nature communications (2023) 141: 3085. . **IHC; KO verified; tested species: mouse**

Proteomic analysis reveals the composition of glutamatergic organelles of auditory inner hair cell.
Cepeda AP, Ninov M, Neef J, Parfentev I, Kusch K, Reisinger E, Jahn R, Moser T, Urlaub H
Molecular & cellular proteomics : MCP (2023) : 100704. . **IHC; tested species: mouse**

A new probe for super-resolution imaging of membranes elucidates trafficking pathways.
Revelo NH, Kamin D, Truckenbrodt S, Wong AB, Reuter-Jessen K, Reisinger E, Moser T, Rizzoli SO
The Journal of cell biology (2014) 2054: 591-606. . **IHC; tested species: mouse**

Selected General References

Differential expression of otoferlin in brain, vestibular system, immature and mature cochlea of the rat.
Schug N et al. Eur. J. Neurosci. (2006) PubMed:17229086

Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse.
Roux I et al. Cell (2006) PubMed:17055430

Auditory neuropathy in patients carrying mutations in the otoferlin gene (OTOF).
Rodríguez-Ballesteros M et al. Hum. Mutat. (2003) PubMed:14635104

Substitutions in the conserved C2C domain of otoferlin cause DFNB9, a form of nonsyndromic autosomal recessive deafness.
Mirghomizadeh F et al. Neurobiol. Dis. (2002) PubMed:12127154

A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness.
Yasunaga S et al. Nat. Genet. (1999) PubMed:10192385

Access the online factsheet including applicable protocols at https://sysy.com/product/178003 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.