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ChT

Cat.No. 216 011; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 μ g purified IgG, lyophilized. Albumin and azide were added for stabilization. For reconstitution add 100 μ l H ₂ O 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 (AP staining) (see remarks) IP: yes ICC: not tested yet IHC: 1: 100 IHC-P: 1: 1000 (see remarks)
Clone	62-2E8
Subtype	IgG1 (κ light chain)
Immunogen	Recombinant protein corresponding to residues near the C-terminus of rat ChT (UniProt Id: Q9JMD7)
Reactivity	Reacts with: monkey, rat (Q9JMD7), mouse (Q8BGY9). Other species not tested yet.
Specificity	K.O. validated
Remarks	WB : To avoid protein aggregation, do not heat samples for SDS-PAGE. IHC-P : Not recommended for rat tissue. Unspecific nuclear signal has been observed using our standard IHC-P protocol.

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

Background

Acetylcholine (ACh) functions as a neurotransmitter in both the central and peripheral nervous systems of all vertebrates, and is the principal neurotransmitter used at the neuromuscular junction. This neurotransmitter is synthesized from choline (Ch) and acetyl-coenzyme A by choline acetyltransferase (ChAT). For this pathway choline is required, which neurons acquire through high-affinity **ch**oline **t**ransporters (**ChT**s). ChT have been found on the presynaptic membrane but also on ACh containing synaptic vesicles.

Selected References for 216 011

Vesicular localization and activity-dependent trafficking of presynaptic choline transporters.

Ferguson SM, Savchenko V, Apparsundaram S, Zwick M, Wright J, Heilman CJ, Yi H, Levey AI, Blakely RD

The Journal of neuroscience: the official journal of the Society for Neuroscience (2003) 2330: 9697-709. . WB, IP, IHC; tested species: mouse

Central Cholinergic Synapse Formation in Optimized Primary Septal-Hippocampal Co-cultures.

Djemil S, Ressel CR, Abdel-Ghani M, Schneeweis AK, Pak DTS

Cellular and molecular neurobiology (2020):.. ICC; tested species: rat

Vesicular acetylcholine transporter (VAChT) over-expression induces major modifications of striatal cholinergic interneuron morphology and function.

Janickova H, Prado VF, Prado MAM, El Mestikawy S, Bernard V Journal of neurochemistry (2017):.. IHC; tested species: mouse

Aberrant trafficking of the high-affinity choline transporter in AP-3-deficient mice.

Misawa H, Fujigaya H, Nishimura T, Moriwaki Y, Okuda T, Kawashima K, Nakata K, Ruggiero AM, Blakely RD, Nakatsu F, Ohno H, et al.

The European journal of neuroscience (2008) 2712: 3109-17.. WB

Distribution of high affinity choline transporter immunoreactivity in the primate central nervous system.

Kus L, Borys E, Ping Chu Y, Ferguson SM, Blakely RD, Emborg ME, Kordower JH, Levey AI, Mufson EJ

The Journal of comparative neurology (2003) 4633: 341-57. . IHC; tested species: monkey

Selected General References

Nerve growth factor regulates the expression of the cholinergic locus and the high-affinity choline transporter via the Akt/PKB signaling pathway.

Madziar B et al. J. Neurochem. (2008) PubMed:18793330

Regulated recycling and plasma membrane recruitment of the high-affinity choline transporter.

Ribeiro FM et al. Eur. J. Neurosci. (2007) PubMed:18088276

The choline transporter resurfaces: new roles for synaptic vesicles?

Ferguson SM et al. Mol. Interv. (2004) PubMed:14993474

Vesicular localization and activity-dependent trafficking of presynaptic choline transporters.

Ferguson SM et al. J. Neurosci. (2003) PubMed:14585997

Purification and reconstitution of the high affinity choline transporter.

Knipper M et al. Biochim. Biophys. Acta (1991) PubMed:1905572

Access the online factsheet including applicable protocols at https://sysy.com/product/216011 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 freezedried) 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 freezethaw cycles.
- Please refer to our tips and hints for subsequent storage of reconstituted antibodies and control peptides and proteins.