SYSY Antibodies

Rudolf-Wissell-Str. 28a
37079 Göttingen, Germany
Phone: +49 551-50556-0
Fax: +49 551-50556-384
E-mail: sales@sysy.com
Web: www.sysy.com

ChT

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

Data Sheet

Reconstitution/ Storage	100 µg purified IgG, lyophilized. Albumin and azide were added for stabilization. For **reconstitution** add 100 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 (AP staining) **IP**: yes **ICC**: not tested yet **IHC**: 1 : 100 **IHC-P**: 1 : 1000
Clone	62-2E8
Subtype	IgG1 (κ light chain)
Immunogen	Recombinant protein corresponding to AA 501 to 580 from rat ChT (UniProt Id: Q9JMD7)
Reactivity	Reacts with: human (Q9GZV3), rat (Q9JMD7), mouse (Q8BGY9). Other species not tested yet.
Specificity	K.O. validated
Remarks	ChT aggregates after boiling, making it necessary to run SDS-PAGE with non-boiled samples.

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 principle 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, Shah S, Brock M, Burke R, Lopez-Coviella I, Nickel AC, Cakal EB, Blusztajn JK, Berse B
Journal of neurochemistry (2008) 1075: 1284-93. .

Regulated recycling and plasma membrane recruitment of the high-affinity choline transporter.
Ribeiro FM, Pinthong M, Black SA, Gordon AC, Prado VF, Prado MA, Rylett RJ, Ferguson SS
The European journal of neuroscience (2007) 2612: 3437-48. .

The choline transporter resurfaces: new roles for synaptic vesicles?
Ferguson SM, Blakely RD
Molecular interventions (2004) 41: 22-37. .

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. .

Purification and reconstitution of the high affinity choline transporter.
Knipper M, Kahle C, Breer H
Biochimica et biophysica acta (1991) 10652: 107-13. .

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 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.