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) (see remarks) **IP**: yes **ICC**: not tested yet **IHC**: 1 : 100 **IHC-P (FFPE)**: 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 (FFPE)**: 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 SYSY antibodies and control proteins/peptides are shipped lyophilized (vacuum freeze-dried). In this form, they remain stable 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. **Do not freeze lyophilized antibodies**. Temperatures below 0°C may impair performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long-term storage of lyophilized fluorophore-conjugates may cause aggregation.
- **Control peptides** should be stored at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- **Do not use frost-free ("no-frost") freezers.** These units periodically warm to remove ice buildup, causing freeze–thaw cycles that can damage antibodies.
- Store vials in areas with minimal temperature fluctuation - preferably toward the back of the freezer, not on the door.
- Aliquot reconstituted antibodies and store at –20°C to –80°C.
- Avoid very small aliquots (<20 µL), as evaporation and adsorption to tube surfaces can reduce antibody concentration and activity.
- Use the smallest practical storage vial to minimize surface area.
- Adding glycerol to a final concentration of 50% prevents freezing at -20°C, allowing storage in liquid form and effectively avoiding freeze–thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C

Monoclonal Antibodies

- **Ascites and hybridoma supernatant:** Store at -20°C to -80°C
  Prolonged storage at 4°C is not recommended, as proteases present in ascites may degrade antibodies.
- **Purified IgG:** Store at -20°C to -80°C
  Adding a carrier protein (e.g., BSA) enhances long-term stability.
  Many SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Polyclonal Antibodies

- **Crude antisera:** Can be stored at 4°C with antimicrobials added, but -20°C to -80°C is preferred
- **Affinity-purified antibodies:** Less stable than antisera; store at -20°C to -80°C
  Adding a carrier protein such as BSA improves long-term stability.
  Most SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Fluorescence-labeled Antibodies

- Store as a liquid with 1:1 (v/v) glycerol at -20°C, and protect from light exposure

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All purified SYSY antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the volume of deionized water specified in the corresponding datasheet. If a larger final volume is desired, first add the recommended amount of water, then adjust with PBS and, if needed, add a stabilizing carrier protein (e.g., BSA) to a final concentration of 2%. Some SYSY antibodies already contain albumin; please take this into account before adding additional carrier protein.

For complete reconstitution, carefully remove the vial cap. After adding water, briefly vortex the solution. To collect the liquid at the bottom of the vial, place the vial inside a 50 ml centrifuge tube padded with paper and centrifuge briefly.

- If desired, small amounts of azide or thimerosal may be added to prevent microbial growth. This is particularly recommended when storing an aliquot at 4°C.
- After reconstitution of fluorescence-labeled antibodies, add glycerol 1:1 (v/v) to achieve a final concentration of 50%. This prevents freezing at –20°C and keeps the antibody in liquid form, effectively avoiding freeze–thaw cycles.
- Glycerol may also be added to unlabeled primary antibodies as a general measure to prevent freeze–thaw damage.
- For further guidance, please refer to our **storage tips** and recommendations for reconstituted antibodies, control peptides, and control proteins.