SYSY Antibodies

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NSF

Cat.No. 123 002; Polyclonal rabbit antibody, 200 µl antiserum (lyophilized)

Data Sheet

Reconstitution/ Storage	200 µl antiserum, lyophilized. For **reconstitution** add 200 µl H_2O, then aliquot and store at -20°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**: 1 : 100 up to 1 : 1000 **IHC**: 1 : 200 **IHC-P (FFPE)**: not tested yet **ELISA**: yes (see remarks)
Immunogen	Synthetic peptide corresponding to AA 733 to 744 from rat NSF (UniProt Id: Q9QUL6)
Reactivity	Reacts with: human (P46459), rat (Q9QUL6), mouse (P46460), hamster. Other species not tested yet.
Matching control	123-0P
Remarks	**ELISA**: The ELISA-protocol for membrane proteins is required. Suitable as detector antibody for sandwich-ELISA. Please refer to the protocol for suitable capture antibodies.

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

Background

N-ethylamide **s**ensitive **f**usion protein **NSF** functions together with SNAPs (soluble NSF attachment proteins) and SNAREs (SNAP receptors) in vesicular transport.
NSF is a homotrimer whose polypeptide subunits are made up of three distinct domains: an amino - terminal domain (N) and two homologous ATP-binding domains (D1 and D2). NSF is an ATPase that dissociates SNARE complexes, such as the core complex composed of synaptobrevin/VAMP, syntaxin 1 and SNAP 25 under ATP hydrolysis. The ability of the D1 domain to hydrolyze ATP is required for NSF activity. The D2 domain is required for trimerization, but its ability to hydrolyze ATP is not absolutely required for NSF function.

Selected References for 123 002

Calcium-triggered acrosomal exocytosis in human spermatozoa requires the coordinated activation of Rab3A and N-ethylmaleimide-sensitive factor.
Michaut M, Tomes CN, De Blas G, Yunes R, Mayorga LS
Proceedings of the National Academy of Sciences of the United States of America (2000) 9718: 9996-10001. . **WB, ICC**

Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins.
Wilhelm BG, Mandad S, Truckenbrodt S, Kröhnert K, Schäfer C, Rammner B, Koo SJ, Claßen GA, Krauss M, Haucke V, Urlaub H, et al.
Science (New York, N.Y.) (2014) 3446187: 1023-8. . **ICC, IHC; tested species: mouse,rat**

Autistic-Like Behavior and Impairment of Serotonin Transporter and AMPA Receptor Trafficking in N-Ethylmaleimide Sensitive Factor Gene-Deficient Mice.
Xie MJ, Iwata K, Ishikawa Y, Nomura Y, Tani T, Murata K, Fukazawa Y, Matsuzaki H
Frontiers in genetics (2021) 12: 748627. . **ICC; tested species: mouse**

Intersectin-Mediated Clearance of SNARE Complexes Is Required for Fast Neurotransmission.
Jäpel M, Gerth F, Sakaba T, Bacetic J, Yao L, Koo SJ, Maritzen T, Freund C, Haucke V
Cell reports (2020) 302: 409-420.e6. . **WB; tested species: mouse**

A connection between reversible tyrosine phosphorylation and SNARE complex-disassembly activity of N-ethylmaleimide-sensitive factor unveiled by the phosphomimetic mutant N-ethylmaleimide-sensitive factor-Y83E.
Ruete MC, Zarelli VEP, Masone D, Paola M, Bustos DM, Tomes CN
Molecular human reproduction (2019) : . . **WB; tested species: human**

Tuning of glutamate, but not GABA, release by an intra-synaptic vesicles APP domain whose function can be modulated by β- or α-secretase cleavage.
Yao W, Tambini MD, Liu X, D'Adamio L
The Journal of neuroscience : the official journal of the Society for Neuroscience (2019) : . . **WB; tested species: mouse**

Pleiotropic effects of alpha-SNAP M105I mutation on oocyte biology: ultrastructural and cellular changes that adversely affect female fertility in mice.
de Paola M, Miró MP, Ratto M, Bátiz LF, Michaut MA
Scientific reports (2019) 91: 17374. . **IHC; tested species: mouse**

Glyoxal as an alternative fixative to formaldehyde in immunostaining and super-resolution microscopy.
Richter KN, Revelo NH, Seitz KJ, Helm MS, Sarkar D, Saleeb RS, D'Este E, Eberle J, Wagner E, Vogl C, Lazaro DF, et al.
The EMBO journal (2018) 371: 139-159. . **ICC; tested species: mouse**

Cortical Granule Exocytosis Is Mediated by Alpha-SNAP and N-Ethilmaleimide Sensitive Factor in Mouse Oocytes.
de Paola M, Bello OD, Michaut MA
PloS one (2015) 108: e0135679. . **WB**

Multi-protein assemblies underlie the mesoscale organization of the plasma membrane.
Saka SK, Honigmann A, Eggeling C, Hell SW, Lang T, Rizzoli SO
Nature communications (2014) 5: 4509. . **ICC; tested species: rat**

Access the online factsheet including applicable protocols at https://sysy.com/product/123002 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.