

## NSF

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

## Data Sheet

Reconstitution/ Storage	200 µl antiserum, lyophilized. For <b>reconstitution</b> add 200 µl H <sub>2</sub> O, 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	<b>WB:</b> 1 : 1000 (AP staining) <b>IP:</b> yes <b>ICC:</b> 1 : 100 up to 1 : 1000 <b>IHC:</b> 1 : 200 <b>IHC_P:</b> yes <b>ELISA:</b> 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	<b>ELISA:</b> Suitable as detector antibody for sandwich-ELISA with cat. no. 123 011 as capture antibody. The ELISA-protocol for membrane proteins is recommended.

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

## Background

**N**-ethylamide sensitive fusion 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, Tomez 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.  
Rüete MC, Zarelli VEP, Masone D, Paola M, Bustos DM, Tomez 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'Adamo 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, Bätz 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-Ethylmaleimide Sensitive Factor in Mouse Oocytes.  
de Paola M, Bello OD, Michaut MA  
PloS one (2015) 108: e0135679. . **WB**

The role of Snapin in neurosecretion: snapin knock-out mice exhibit impaired calcium-dependent exocytosis of large dense-core vesicles in chromaffin cells.  
Tian JH, Wu ZX, Unzicker M, Lu L, Cai Q, Li C, Schirra C, Matti U, Stevens D, Deng C, Rettig J, et al.  
The Journal of neuroscience : the official journal of the Society for Neuroscience (2005) 2545: 10546-55. . **WB**

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 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 at 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.