

## NP-EGTA

Cat.No. 510 006; , 1 mg photolabile calcium-chelator

### Data Sheet

Reconstitution/ Storage	1 mg o-nitrophenyl EGTA tetrapotassium salt dissolved in 50 µl H <sub>2</sub> O (= 30.5 mM). <a href="#">HPLC analysis</a> Spin down and store at -20° C. Protect material from light always.
For detailed information, see back of the data sheet.	
Name	6,9-Dioxa-3,12-diazatetradecanedioic acid, 3,12-bis(carboxymethyl)-4-(2-nitrophenyl)-tetrapotassium salt.
Molecular formula	C <sub>20</sub> H <sub>23</sub> K <sub>4</sub> N <sub>3</sub> O <sub>12</sub> . chemical structure
Molecular weight	653.81
Extinction coefficient	$\epsilon = 5.52 \times 10^3 \text{ M}^{-1} \times \text{cm}^{-1}$ at 260 nm in Ca <sup>2+</sup> free 40 mM HEPES / 100 mM KCl buffer at pH 7.2.
Photolysis quantum yield	0.23

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

## Background

Calcium is the most important signalling molecule inside cells. It is involved in the regulation of neurotransmission, gene-expression, muscle contraction and many more.  
**o-nitrophenyl EGTA (NP-EGTA)** is a photolabile Ca<sup>2+</sup> chelator that is highly specific for Ca<sup>2+</sup> ions and unaffected by physiological Mg<sup>2+</sup> concentrations. Photolysis by illumination with UV-light decreases the affinity of NP-EGTA for Ca<sup>2+</sup> ions ~ 12,500-fold and the Ca<sup>2+</sup> ions become physiologically available. By this approach regulatory effects of calcium on cellular processes can be studied.

## Selected References for 510 006

Rab3 proteins involved in vesicle biogenesis and priming in embryonic mouse chromaffin cells.  
Schonn JS, van Weering JR, Mohrmann R, Schlüter OM, Südhof TC, de Wit H, Verhage M, Sørensen JB  
Traffic (Copenhagen, Denmark) (2010) 1111: 1415-28. .

Analysis of neurotransmitter release mechanisms by photolysis of caged Ca<sup>2+</sup> in an autaptic neuron culture system.  
Burgalossi A, Jung S, Man KN, Nair R, Jockusch WJ, Wojcik SM, Brose N, Rhee JS  
Nature protocols (2012) 77: 1351-65. .

Fast vesicle fusion in living cells requires at least three SNARE complexes.  
Mohrmann R, de Wit H, Verhage M, Neher E, Sørensen JB  
Science (New York, N.Y.) (2010) 3306003: 502-5. .

SNARE protein recycling by αSNAP and βSNAP supports synaptic vesicle priming.  
Burgalossi A, Jung S, Meyer G, Jockusch WJ, Jahn O, Taschenberger H, O'Connor VM, Nishiki T, Takahashi M, Brose N, Rhee JS, et al.  
Neuron (2010) 683: 473-87. .

Progression of diet-induced diabetes in C57BL6J mice involves functional dissociation of Ca<sup>2+</sup> channels from secretory vesicles.  
Collins SC, Hoppa MB, Walker JN, Amisten S, Abdulkader F, Bengtsson M, Fearnside J, Ramacheya R, Toye AA, Zhang Q, Clark A, et al.  
Diabetes (2010) 595: 1192-201. .

Role of the synaptobrevin C terminus in fusion pore formation.  
Ngatchou AN, Kisler K, Fang Q, Walter AM, Zhao Y, Bruns D, Sørensen JB, Lindau M  
Proceedings of the National Academy of Sciences of the United States of America (2010) 10743: 18463-8. .

Synaptotagmin interaction with SNAP-25 governs vesicle docking, priming, and fusion triggering.  
Mohrmann R, de Wit H, Connell E, Pinheiro PS, Leese C, Bruns D, Davletov B, Verhage M, Sørensen JB  
The Journal of neuroscience : the official journal of the Society for Neuroscience (2013) 3336: 14417-30. .

Doc2b synchronizes secretion from chromaffin cells by stimulating fast and inhibiting sustained release.  
Pinheiro PS, de Wit H, Walter AM, Groffen AJ, Verhage M, Sørensen JB  
The Journal of neuroscience : the official journal of the Society for Neuroscience (2013) 3342: 16459-70. .

Phosphatidylinositol 4,5-bisphosphate optical uncaging potentiates exocytosis.  
Walter AM, Müller R, Tawfik B, Wierda KD, Pinheiro PS, Nadler A, McCarthy AW, Ziolkiewicz I, Kruse M, Reither G, Rettig J, et al.  
eLife (2017) 6: . .

## Selected General References

Examining synaptotagmin 1 function in dense core vesicle exocytosis under direct control of Ca<sup>2+</sup>.  
Sørensen JB et al. J. Gen. Physiol. (2003) PubMed:12939392

Protein kinase C-dependent phosphorylation of synaptosome-associated protein of 25 kDa at Ser187 potentiates vesicle recruitment.  
Nagy G et al. J. Neurosci. (2002) PubMed:12417653

Access the online factsheet including applicable protocols at <https://sysy.com/product/510006> 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.