

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 ₂ O (= 30.5 mM). HPLC analysis 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 ₂₀ H ₂₃ K ₄ N ₃ O ₁₂ . 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 ²⁺ 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 Ca²⁺ chelator that is highly specific for Ca²⁺ ions and unaffected by physiological Mg²⁺ concentrations. Photolysis by illumination with UV-light decreases the affinity of NP-EGTA for Ca²⁺ ions ~ 12,500-fold and the Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ channels from secretory vesicles.
Collins SC, Hoppa MB, Walker JN, Amisten S, Abdulkader F, Bengtsson M, Fearnside J, Ramracheya 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 Ca²⁺.
Sørensen JB, Fernández-Chacón R, Südhof TC, Neher E
The Journal of general physiology (2003) 1223: 265-76. .

Protein kinase C-dependent phosphorylation of synaptosome-associated protein of 25 kDa at Ser187 potentiates vesicle recruitment.
Nagy G, Matti U, Nehring RB, Binz T, Rettig J, Neher E, Sørensen JB
The Journal of neuroscience : the official journal of the Society for Neuroscience (2002) 2221: 9278-86. .

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