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

Tight junction protein ZO-3

Cat.No. 505 005; Polyclonal Guinea pig antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For **reconstitution** add 50 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 up to 1 : 2000 (AP staining) **IP**: not tested yet **ICC**: 1 : 500 up to 1 : 1000 (see remarks) **IHC**: 1 : 1000 (see remarks) **IHC-P (FFPE)**: 1 : 1000 up to 1 : 2000 **IHC-Fr**: 1 : 500 up to 1 : 1000 (see remarks)
Immunogen	Recombinant protein corresponding to residues near the carboxy terminus of mouse Tight junction protein ZO-3 (UniProt Id: Q9QXY1)
Reactivity	Reacts with: mouse (Q9QXY1), rat (D3Z8G7), dog (O62683). No signal: human (O95049). Other species not tested yet.
Remarks	**ICC**: Methanol fixation is recommended. **IHC**: Antigen retrieval with citrate buffer pH 6 is required. **IHC-Fr**: Methanol-acetone fixation is recommended.

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

Background

ZO-3 is a 130 kDa protein identified by co-immunoprecipitation with ZO-1 from Madin-Darby canine kidney (MDCK) cells. (1)
ZO-3 is a member of the membrane-associated guanylate kinase-like protein (MAGUK) family associated with intracellular junctions. (2)
The zona occludens proteins ZO-1, -2 and -3 (also known as TJP1, 2 and 3) are peripheral membrane adaptor proteins that link transmembrane junction proteins such as occludin and claudin to the actin cytoskeleton (3).

Selected General References

ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin.
Haskins J et al. J Cell Biol (1998) PubMed:9531559

Epithelial tight junctions, gene expression and nucleo-junctional interplay.
Matter K et al. J Cell Sci (2007) PubMed:17452622

Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins.
Itoh M et al. J Cell Biol (1999) PubMed:10601346

Access the online factsheet including applicable protocols at https://sysy.com/product/505005 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.