SYSY Antibodies

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CD25

Cat.No. 551 003; Polyclonal rabbit 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 (AP staining) **IP**: not tested yet **ICC**: not tested yet **IHC**: 1 : 500 up to 1 : 1000 (see remarks) **IHC-P (FFPE)**: 1 : 1000
Immunogen	Synthetic peptide corresponding to residues surrounding AA131 of mouse CD25 (UniProt Id: P01590)
Reactivity	Reacts with: mouse (P01590). No signal: human (P01589), rat. Other species not tested yet.
Remarks	**IHC**: Antigen retrieval with citrate buffer pH 6 is required.

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

Background

Interleukin-2 receptor alpha (IL-2Rα), also known as CD25, is a type I transmembrane glycoprotein that constitutes the α-chain of the trimeric interleukin-2 (IL-2) receptor complex. Structurally, CD25 comprises a large extracellular domain responsible for IL-2 binding, a single transmembrane region, and a short cytoplasmic tail that lacks intrinsic signaling motifs. Consequently, CD25 does not directly mediate intracellular signal transduction but primarily functions to increase the affinity of the IL-2 receptor for its ligand (1). During thymic T-cell development, CD25 expression marks intermediate CD4−CD8− double-negative thymocyte populations, particularly the DN2 and DN3 stages (2). CD25 is also constitutively expressed at variable levels on regulatory T cells (Tregs) (3,4). High CD25 expression on Foxp3 Tregs is associated with potent immunosuppressive activity and has been linked to poor prognosis in colorectal cancer (5). In contrast, Foxp3 Tregs residing in the small intestinal epithelium markedly downregulate CD25 expression and persist independently of IL-2 while retaining strong suppressive function (4).
Beyond its membrane-bound form, CD25 also exists as a soluble receptor generated through proteolytic shedding. Circulating levels of soluble CD25 are dysregulated in numerous pathological conditions, including cancer, inflammatory diseases, and autoimmune disorders, highlighting its relevance as a biomarker of immune activation (1).

Selected General References

The soluble IL-2 receptor α/CD25 as a modulator of IL-2 function.
Lokau J et al. Immunology (2024) PubMed:38037265

Spatial analysis and CD25-expression identify regulatory T cells as predictors of a poor prognosis in colorectal cancer.
Bergsland CH et al. Mod Pathol (2022) PubMed:35484226

The small intestine epithelium exempts Foxp3+ Tregs from their IL-2 requirement for homeostasis and effector function.
Prakhar P et al. JCI Insight (2021) PubMed:34747370

Developmental and molecular characterization of emerging beta- and gammadelta-selected pre-T cells in the adult mouse thymus.
Taghon T et al. Immunity (2006) PubMed:16413923

Natural regulatory CD4 T cells expressing CD25.
Papiernik M et al. Microbes Infect (2001) PubMed:11564442

Access the online factsheet including applicable protocols at https://sysy.com/product/551003 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.