SYSY Antibodies

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CD28

Cat.No. 555 003; 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 : 500 (fluorescent detection) **IP**: not tested yet **ICC**: not tested yet **IHC**: not tested yet **IHC-P (FFPE)**: yes (see remarks)
Immunogen	Synthetic peptide corresponding to residues near the carboxy terminus of human CD28 (UniProt Id: P10747)
Reactivity	Reacts with: human (P10747). Other species not tested yet.
Remarks	**IHC-P (FFPE)**: Antigen retrieval with Tris-EDTA buffer pH 9 is recommended for chromogenic detection. For chromogenic detection, an optimized AGR time of 20 minutes is recommended for best results.

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

Background

CD28 is a central co-stimulatory receptor required for optimal T-cell activation, expansion, and survival. Constitutively expressed on most naïve T-cells, CD28 binds the B7 family ligands CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells, delivering the “second signal” necessary to complement T-cell receptor (TCR) recognition of antigen and prevent anergy (1, 2). Upon ligation, CD28 amplifies intracellular signaling pathways including phosphoinositide 3-kinase (PI3K), Akt, NF-κB, and MAPK, thereby enhancing interleukin-2 (IL-2) production, cell-cycle progression, metabolic fitness, and resistance to apoptosis (3, 4). In addition to its role in normal immune responses, dysregulated CD28 signaling contributes to autoimmunity, chronic inflammation, and transplant rejection, while its manipulation has become highly relevant in cancer immunotherapy and immune modulation (5). The importance of CD28 is also underscored in chimeric antigen receptor (CAR) T-cell design, where incorporation of the CD28 intracellular signaling domain promotes robust effector activity and functional persistence (6, 7).

Selected General References

CTLA-4 is a second receptor for the B cell activation antigen B7.
Linsley PS et al. J Exp Med (1991) PubMed:1714933

CD28 Costimulation: From Mechanism to Therapy.
Esensten JH et al. Immunity (2016) PubMed:27192564

The basic principles of chimeric antigen receptor design.
Sadelain M et al. Cancer Discov (2013) PubMed:23550147

An enigmatic tail of CD28 signaling.
Boomer JS et al. Cold Spring Harb Perspect Biol (2010) PubMed:20534709

Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor.
Maher J et al. Nat Biotechnol (2002) PubMed:11753365

CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones.
Harding FA et al. Nature (1992) PubMed:1313950

Regulation of interleukin-2 gene enhancer activity by the T cell accessory molecule CD28.
Fraser JD et al. Science (1991) PubMed:1846244

Access the online factsheet including applicable protocols at https://sysy.com/product/555003 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.