SYSY HistoSure

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CD86 (B7.2) mouse specific

Cat.No. HS-466 003; 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 ₂ O 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 : 250 IP: not tested yet ICC: not tested yet IHC: 1 : 500 (see remarks) IHC-P: 1 : 400
Immunogen	Synthetic peptide corresponding to AA 291 to 309 from mouse Cd86 (UniProt Id: P42082)
Reactivity	Reacts with: mouse (P42082). No signal: human (P42081), rat. Other species not tested yet.
Remarks	IHC : Heat-mediated antigen retrieval (in citrate buffer pH 6) is required for immunohistochemical staining of PFA fixed tissues. Methanol fixation is required for immunohistochemical staining of fresh frozen tissues.

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

Background

CD86 (Cluster of Differentiation 86, also known as B7.2) belongs to the B7 family of immuneregulatory cell-surface protein ligands (1). CD86 and the genetically closely linked CD80 protein (also known as B7.1) are expressed by antigen presenting cells and provide costimulatory signals necessary for T cell activation and tolerance via interaction with CD28 and cytotoxic T-lymphocyte antigen 4 (CTLA-4) expressed on T-cells. However, CD80 and CD86 have non-equivalent roles in immune modulation: CD86 is the dominant ligand for proliferation and survival of regulatory T cells (Tregs) (2) and shows in comparison with CD80 very high efficiency at increasing T cell killing capacity (3). CD86 is expressed only at low levels on resting B cells, dendritic cells and macrophages; activation results in enhanced CD86 expression (Collins et al., 2005). In the CNS, CD86 upregulation is a marker of activated pro-inflammatory M1 microglia (4). In oncology research, CD86 is a biomarker to phenotypically differentiate classically activated M1 macrophages from alternatively activated M2 macrophages in the tumor microenvironment (5).

Selected General References

The B7 family of immune-regulatory ligands. Collins M et al. Genome Biol (2005) PubMed:15960813

Tumor-associated macrophages: potential therapeutic strategies and future prospects in cancer. Li C et al. J Immunother Cancer (2021) PubMed:33504575

CD86 Is a Selective CD28 Ligand Supporting FoxP3+ Regulatory T Cell Homeostasis in the Presence of High Levels of CTLA-4. Halliday N et al. Front Immunol (2020) PubMed:33363541

Overview of General and Discriminating Markers of Differential Microglia Phenotypes. Jurga AM et al. Front Cell Neurosci (2020) PubMed:32848611

Efficiency of T-cell costimulation by CD80 and CD86 cross-linking correlates with calcium entry. Thiel M et al. Immunology (2010) PubMed:19824921

Access the online factsheet including applicable protocols at <u>https://sysy-histosure.com/product/HS-466003</u> 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 freezedried) 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 freezethaw cycles.
- Please refer to our **tips and hints for subsequent storage** of reconstituted antibodies and control peptides and proteins.