Synaptic Systems

Rudolf-Wissell-Str. 28
37079 Göttingen, Germany
Phone: +49 551-50556-0
Fax: +49 551-50556-384
E-mail: sales@sysy.com
Web: www.sysy.com

CD 4

Cat.No. 360 004; Polyclonal Guinea pig antibody, 100 µl antiserum (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µl antiserum, lyophilized. For reconstitution add 100 µl H_2O, then aliquot and store at -20°C until use.
Applications	**WB**: not tested yet **IP**: not tested yet **ICC**: not tested yet **IHC**: 1 : 500 **IHC-P/FFPE**: 1 : 500
Immunogen	Recombinant protein corresponding to AA 27 to 394 from mouse CD4 (UniProt Id: P06332)
Reactivity	Reacts with: mouse (P06332). No signal: human (P01730), rat (P05540). Other species not tested yet.
Specificity	Specific for CD 4.

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

CD 4 (**c**luster of **d**ifferentiation **4**) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells.
CD 4 is a co-receptor that assists the T cell receptor (TCR) in communicating with an antigen-presenting cell.

Selected General References

A gene-rich cluster between the CD4 and triosephosphate isomerase genes at human chromosome 12p13.
Ansari-Lari MA, Muzny DM, Lu J, Lu F, Lilley CE, Spanos S, Malley T, Gibbs RA
Genome research (1996) 6(4): 314-26.

Crystal structure of domains 3 and 4 of rat CD4: relation to the NH2-terminal domains.
Brady RL, Dodson EJ, Dodson GG, Lange G, Davis SJ, Williams AF, Barclay AN
Science (New York, N.Y.) (1993) 260(5110): 979-83.

CD4 and CD8 subsets defined by dual-color cytofluorometry which distinguish symptomatic from asymptomatic blood donors seropositive for human immunodeficiency virus.
Prince HE, Arens L, Kleinman SH
Diagnostic and clinical immunology (1987) 5(4): 188-93.

Function of the CD4 and CD8 molecules on human cytotoxic T lymphocytes: regulation of T cell triggering.
Fleischer B, Schrezenmeier H, Wagner H
Journal of immunology (Baltimore, Md. : 1950) (1986) 136(5): 1625-8.

Synaptic Systems

Rudolf-Wissell-Str. 28
37079 Göttingen, Germany
Phone: +49 551-50556-0
Fax: +49 551-50556-384
E-mail: sales@sysy.com
Web: www.sysy.com

IHC protocol for CD 4 antibody

cat. no. 360 004, 100 µl Guinea pig antiserum

Reagents needed:

- Xylene
- Ethanol
- **Target Retrieval Solution:** 10mM citrate buffer, 0.05% Tween 20, pH 6.0
- **PBS:** pH 7.4
- **TBST:** 50mM Tris, 150mM NaCl, 0.05% Tween 20, pH 7.6
- **Hydrogen peroxidase:** 3% H2O2 in PBS
- Avidin/Biotin block
- **Blocking solution:** 2.5% normal goat serum in PBS (e.g. ready-to-use, Vector Laboratories) or serum-free protein block (e.g. Dako X0909)
- **Incubation buffer:** 2.5% normal goat serum in PBS (e.g. ready-to-use, Vector Laboratories) or antibody diluent (e.g. Dako S2022)
- **Guinea pig anti-mouse CD 4** diluted in incubation buffer at 1:250 – 1:500
- **Biotinylated anti-guinea pig** (e.g. Jackson 106-065-003) diluted in incubation buffer at 5µg/ml
- ABC Kit HRP
- DAB working solution
- Counterstain (e.g. Mayer's Hemalaun)
- Mounting medium

Material needed:

- Heat steamer (e.g. BRAUN MultiGourmet)
- Thin-walled plastic staining jars (e.g. Sakura)

Deparaffinization and rehydration:

1. Incubate sections with xylene twice for 5min.
2. Rehydrate sections through a series of ethanol concentration (2min each): 2 x 100% EtOH, 90% EtOH, 80% EtOH, 2 x 70% EtOH.
3. Rinse sections in dH2O.
4. Keep the slides in PBS until ready to perform antigen retrieval.

Staining procedure:

1. **Antigen Retrieval:** Heat the steamer with the staining jar filled with the target retrieval buffer to ~ 97-100°C and transfer the sections into the preheated antigen retrieval buffer.
   Wait until the temperature reaches again 97°C and start the antigen retrieval time: **30min**.
   Remove the staining jar and allow the slides to cool down for **20min** to ~ 60°C.
2. Rinse slides in PBS (3 x 1min).
3. **Blocking endogenous peroxidase activity:** Incubate slides for **5min** in 3 % H2O2.
4. Wash slides in PBS (2 x 1min).
5. Wash slides in TBST (1 x 2min).
6. Perform an **Avidin/Biotin blocking step** in case of presence of endogenous protein-associated biotin in the tissue.
7. **Protein Block:** Incubate slides with protein block (2.5% NGS: 20min; serum-free block: 10min) in a humified chamber.
8. **Primary Antibody:** Remove blocking solution by tilting and incubate slides for 60min with the primary antibody diluted in incubation buffer at RT in a humified chamber.
9. Wash slides in TBST (3 x 2min).
10. **Secondary antibody:** Incubate slides for 30min with biotinylated anti-guinea pig secondary antibody diluted in incubation buffer at RT in a humified chamber.
11. Wash slides in TBST (3 x 2min).
12. **ABC Reagent:** Incubate slides for 30min with ABC Reagent at RT in a humified chamber.
13. Wash slides in TBST (3 x 2min).
14. **DAB chromogen:** Incubate in peroxidase substrate until desired staining intensity is reached.
15. Rinse slides in dH2O.
16. Counterstain (e.g. Mayer's Hemalaun).
17. Dehydrate tissue sections in a graded series of ethanol concentration (0% to 100%) clear in xylene and mount using a non-aqueous mounting medium.