

Anti-CD52 antibody, rabbit polyclonal, KO-Validated

73-030 100 μ l

Validation: Specificity validated with KO mouse (Fig.2)

Storage: Shipped at 4°C or at -20°C. Upon arrival, spin-down and store at -20°C..

Reactivity: Mouse. Likely to react with rat due to the sequence identity.

Applications:

1. Western blotting (1/1,000 dilution)
2. Immunohistchemistry-P (1/100 dilution)
3. Immunofluorescence staining (1/100 dilution)

Immunogen: Synthetic peptide corresponding to 29-48 amino acids of mouse CD52, C-AASGTNKNSTSTKKTPLKSG, conjugated with KLH

Form: Whole rabbit antiserum added with 0.1% sodium azide.

Key words: CD52, CAMPATH-1 antigen, Lymphocyte differentiation antigen B7, Cell membrane, GPI-anchor, Glycoprotein.

Function: CD52 may play a role in carrying and orienting carbohydrate, as well as having a more specific role. Expressed on lymphohematopoietic tissues, including thymus, spleen, and bone marrow, but not in liver, kidney, and brain.

Molecular mass: 7,798 Da with 74 amino acids. In mature form, propeptide is removed, and GPI anchored and glycosylated.

Database Links: [uniprot/Q64389](http://uniprot.org/Q64389) Mouse CD52 [Gene ID 23833](http://ncbi.nlm.nih.gov/RefSeq/NC_007096.3/gene/23833) Mouse CD52

Reference: This antibody was described in Ref.1 and used in the following publications. Yamaguchi R et al. (2008) Cd52, known as a major maturation-associated sperm membrane antigen secreted from the epididymis, is not required for fertilization in the mouse. [Genes Cells](http://www.genesandcells.com). 13:851-61.WB.

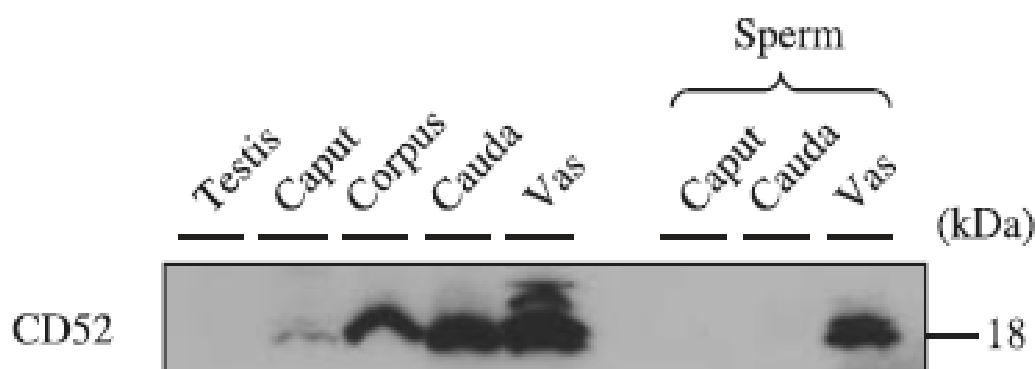


Fig.1 Western blotting analysis of CD52 expression in various tissues with anti-CD52 antibody.

Testes, male reproductive ducts and sperm protein were extracted with lysis buffer containing Triton X-100 and subjected to western blot analysis. Western blots containing equal amounts of tissue proteins (30 μ g) and sperm protein (10 μ g) were reacted with anti-CD52 antibody at 1/1,000 dilution.

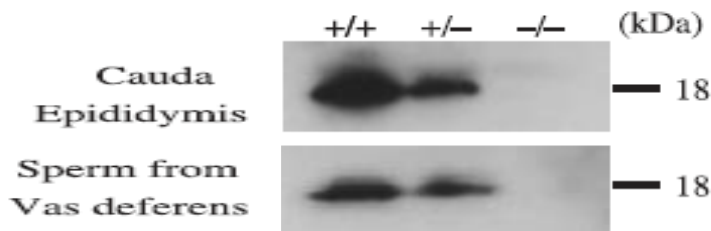


Fig.2. Western blot analysis of CD52 in cauda epididymal lysates and sperm lysates of wild type and CD52 deficient mice. *Cd52^{+/+}* (+/+), *Cd52^{+/-}* (+/-), and *Cd52^{-/-}* (-/-).

Cauda epididymis and sperm from vas deferens were lysed in lysis buffer containing 1% TritonX-100. Proteins (30 μ g for cauda epididymis and 10 μ g for sperm) were analyzed by western blotting with anti-CD52 antibody at 1/1,000 dilution.

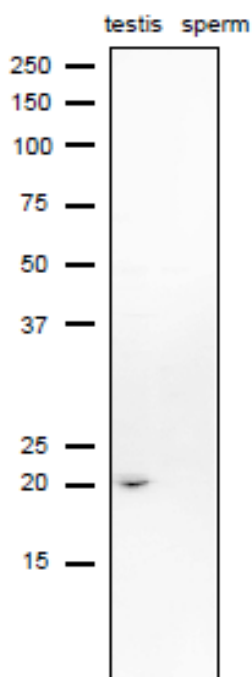


Fig.3. Western blot analysis of CD52 in lysates of mouse testis and sperm with anti-CD52 antibody.

Proteins in the lysates (10 μ g) are separated on SDS-PAGE (10-20% gradient) and electro-blotted to PVDF membrane. The membrane was reacted with anti-CD52 antibody at 1/1,000 dilution. As the second antibody, anti-rabbit IgG antibody conjugated with HRP (ab97051) was used at 1/10,000 dilution. The numbers on the right are positions of protein size markers shown in kDa.

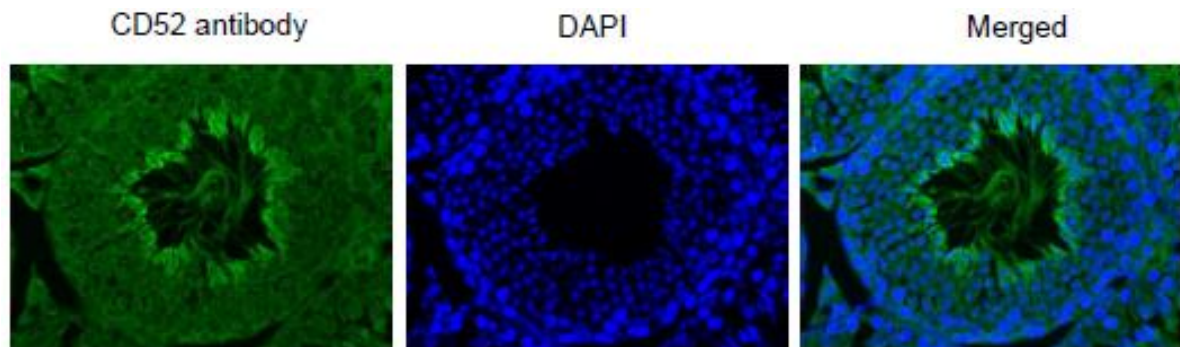


Fig.4. Immunohistochemistry of mouse testis using anti-CD52 antibody.

Formalin-fixed and paraffin-embedded mouse testis

Deparaffinization by LemosolRA (#122-03991, Wako, Osaka)

Rehydration 100% Et-OH, 95%, 90%, 70%, DW

Antigen retrieval Histo/Zyme (Cat.# k046; Diagnostic BioSystems)

Washing PBST (0.25% triton X-100/PBS-)

Blocking 10 % FBS / PBST 30 min

1st antibody 1/100 dilution in PBS- 4°C O/N

Washing PBS-

2nd antibody 1,000 dilution, 60 min (Alexa Flour-488 goat anti-rabbit IgG (H&L))

Washing PBS- 5 min, 3 times

DAPI 1.0µg/mL DAPI in TBS 10 min

Mount ImmunoSelect Antifading Mounting Medium (SCR-38447; Dianova)

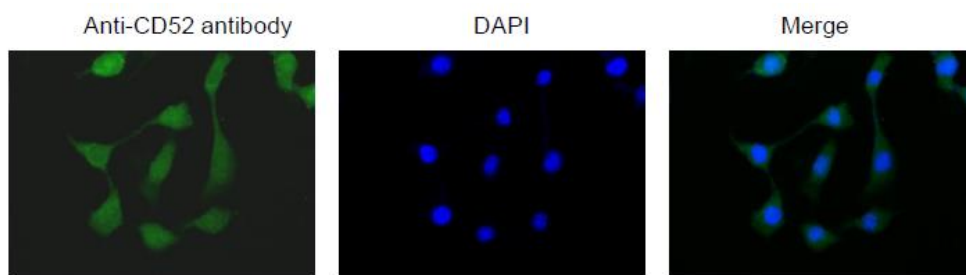


Fig. 5 Immunofluorescence staining of CD52 in NIH3T3 cells with anti-CD52

antibody. The cells were fixed in 4% paraaformaldehyde over night

Permeabilization in 0.25% Triton X-100/PBS for 10 min

Blocking in 1.5% BSA/PBS for 30 min

1st antibodies diluted 1/100 by blocking buffer and incubated over night

2nd antibody, goat, anti-mouse IgG conjugated with Alex 488 (1/1000 dilution).

Nuclei were stained with DAPI