

## Anti-CD52 antibody, rabbit serum, KO-Validated

73-030 100 µl

**Shipping and Storage:** Shipped at 4°C or -20°C and store at -20°C.

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

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

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

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

### Applications:

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

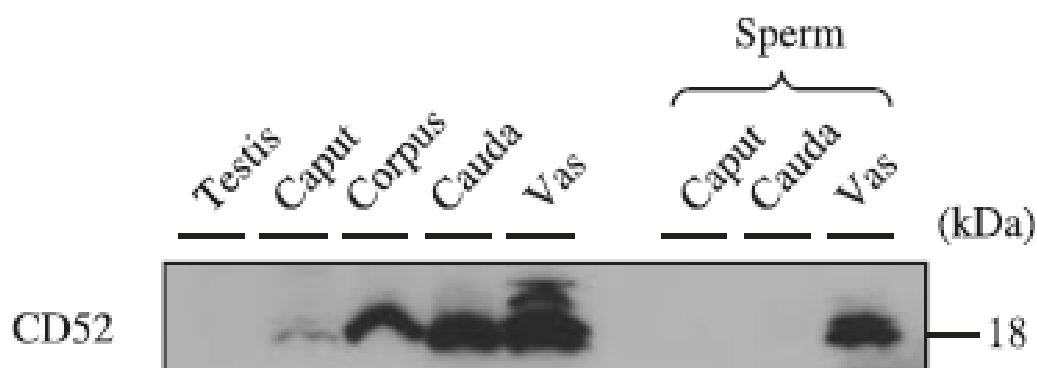
**Key words:** CD52, CAMPATH-1 antigen, Lymphocyte differentiation antigen B7, Cell membrane, GPI-anchor, Glycoprotein.

**Background:** 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.

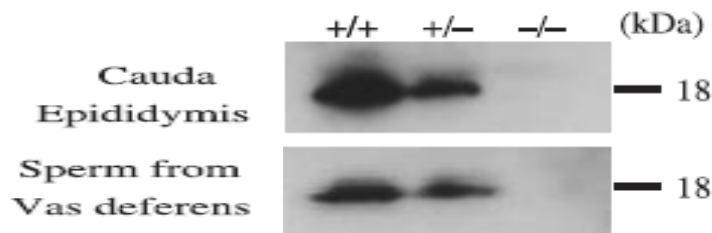
**Database Links:** [uniprot/Q64389](http://uniprot.org/Q64389) Mouse CD52 [Gene ID 23833](http://ncbi.nlm.nih.gov/RefSeq/NC_007094.3/gene/23833) Mouse CD52

**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*. 13:851-61. PMID: [18782223](https://pubmed.ncbi.nlm.nih.gov/18782223/) **WB (mouse).**



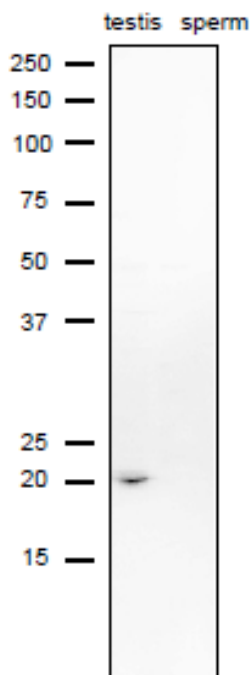
**Fig.1** Western blotting analysis of CD52 expression in various tissues with anti-CD52 antibody.

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 µg) and sperm protein (10 µg) were reacted with anti-CD52 antibody at 1/1,000 dilution.



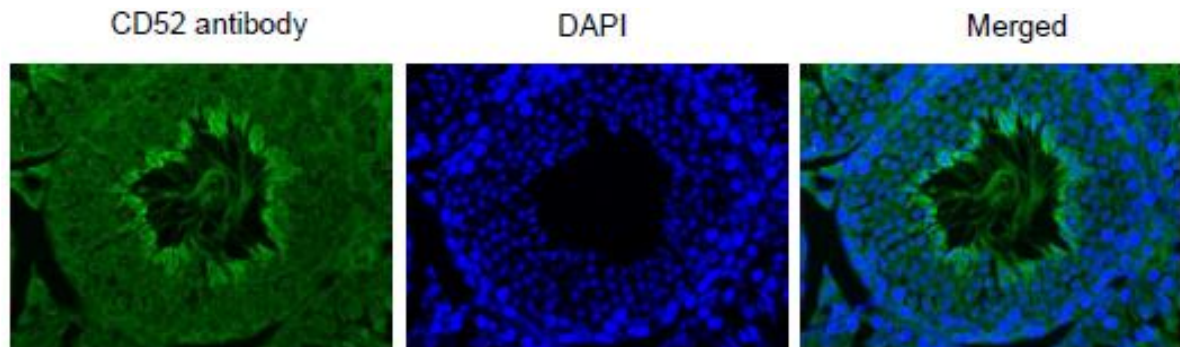
**Fig.2. Western blot analysis of CD52 in cauda epididymal lysates and sperm lysates of wild type and CD52 deficient mice.** *Cd52<sup>+/+</sup>* (+/+), *Cd52<sup>+/-</sup>* (+/-), and *Cd52<sup>-/-</sup>* (-/-).

Cauda epididymis and sperm from vas deferens were lysed in lysis buffer containing 1% TritonX-100. Proteins (30  $\mu$ g for cauda epididymis and 10  $\mu$ 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  $\mu$ 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

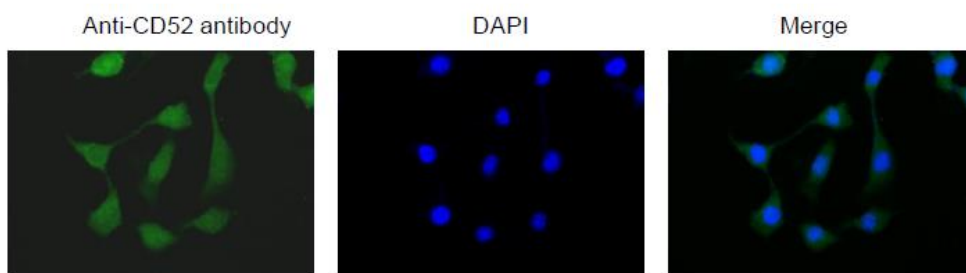
Washing PBS-

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