

## **Anti-CD52 antibody, rabbit polyclonal, KO-Validated**

Cat.#73-030, Size:100 µl

### **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.

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

### **Specifications:**

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

**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.

**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.

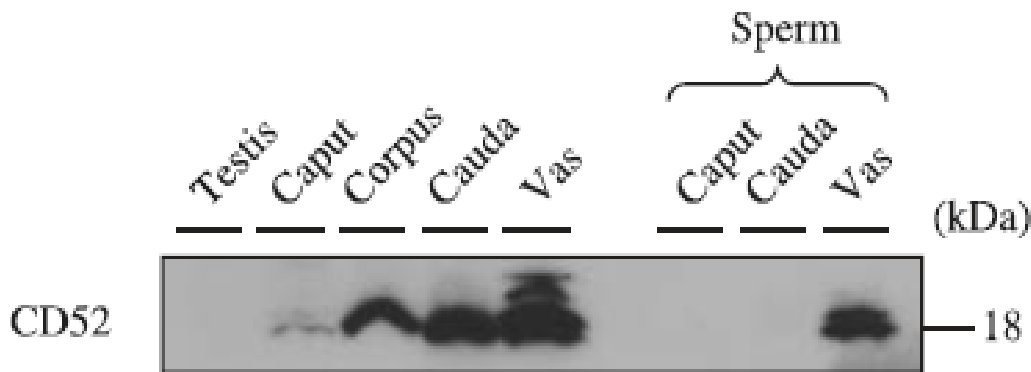
### **Applications:**

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

**Database Links:** [uniprot/Q64389](https://uniprot.org/Q64389) Mouse CD52      [Gene ID 23833](https://ncbi.nlm.nih.gov/nuccore/23833) Mouse CD52

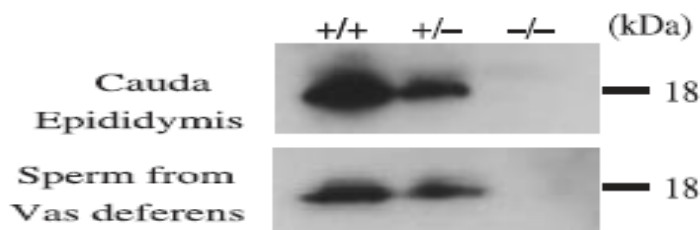
**Reference:** This antibody was described in Ref.1 and used in the following publications.

1. 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.**WB**.



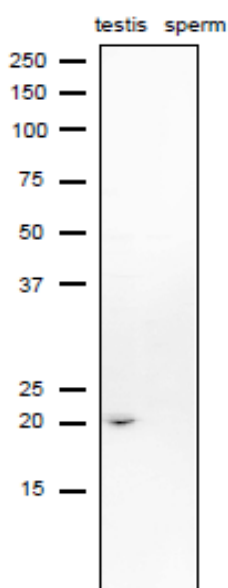
**Fig.1 Western blotting analysis of CD52 expression in various tissues with anti-CD52 antibody.**

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

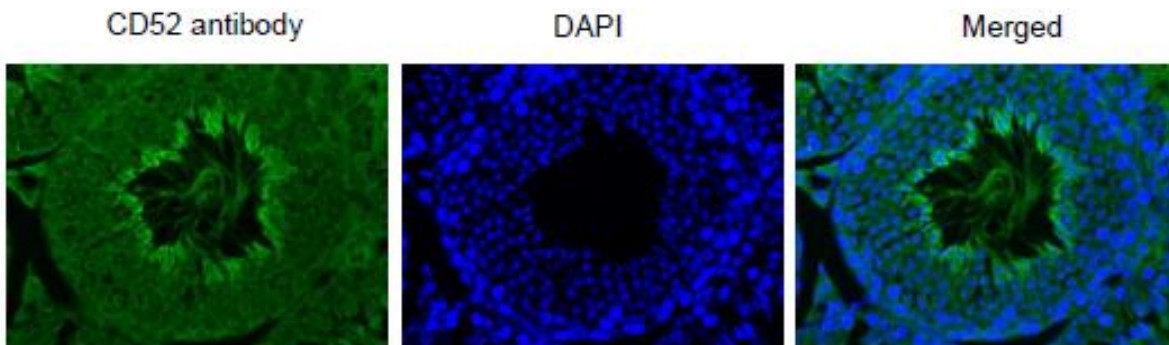


**Fig.2. Western blot analysis of CD52 in cauda epididymal lysates and sperm lysates of wild type and CD52 deficient mice.** *Cd52<sup>+/+</sup>* (+/+), *Cd52<sup>+/-</sup>* (+/-), and *Cd52<sup>-/-</sup>* (-/-).

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

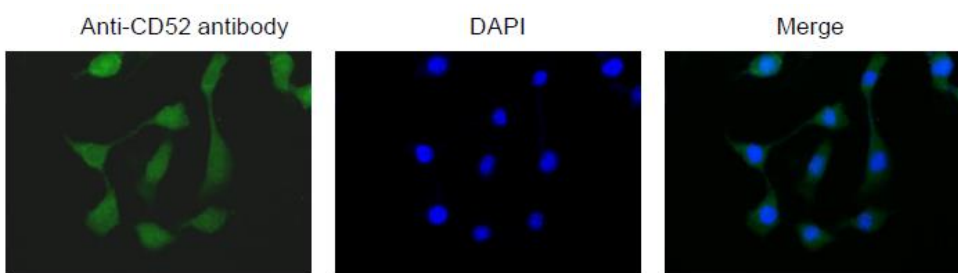
Washing PBS-

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 overnight.

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 withAlex 488 (1/1000 dilution).

Nuclei were stained with DAPI.