Anti-CD52 antibody, rabbit polyclonal

Product code	73-710
Size	100 µg
Storage	-20℃
Concentration	1mg/ml
Buffer	PBS- with 50 % glycerol
Purity	Purified IgG fraction with protein A from rabbit antiserum (73-030).
Immunogen	Synthetic peptide corresponding to 29-48 amino acids of mouse CD52, C-AASGTNKNSTSTKKTPLKSG , conjugated with KLH
Isotype	Rabbit IgG
Reactivity	Mouse. Likely to react with rat due to the sequence identity.
Special notes	Validation: Specificity validated with KO mouse (Fig.2)
Application	1. Western blotting (1/1,000 dilution) 2. Immunohistchemistry-P (1/100 dilution) 3. Immunofluorescence staining (1/100 dilution)
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.
Data Link	uniprot/Q64389 Mouse CD52 Gene ID 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. PMID: 18782223 **WB (mouse)**.
Related Products	73-030 Anti-CD52 antibody, rabbit serum

Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.

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Data Images: 73-710 Anti-CD52 antibody, rabbit polyclonal

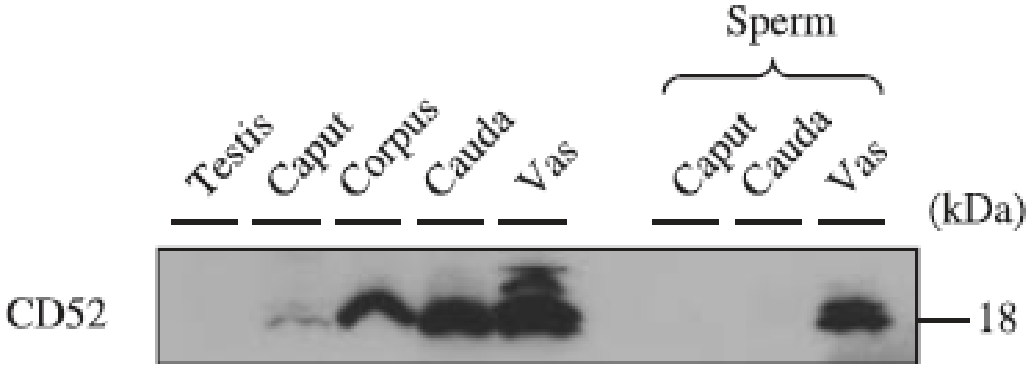


Fig.1 Western blotting analysis of CD52 expression in various tissues with anti-CD52 antibody.

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.

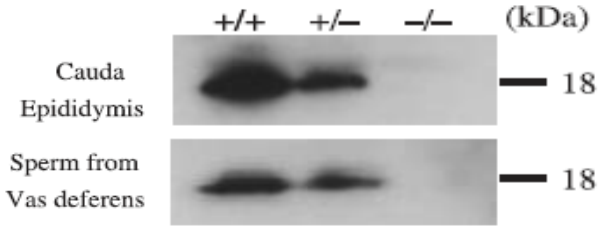


Fig.2. Western blot analysis of CD52 in cauda epididymal lysates and sperm lysates of wild type and CD52 deficient mice. *Cd52*$^{+/+}$ (+/+), *Cd52*$^{+/-}$ (+/–), and *Cd52*$^{-/-}$(–/–).

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

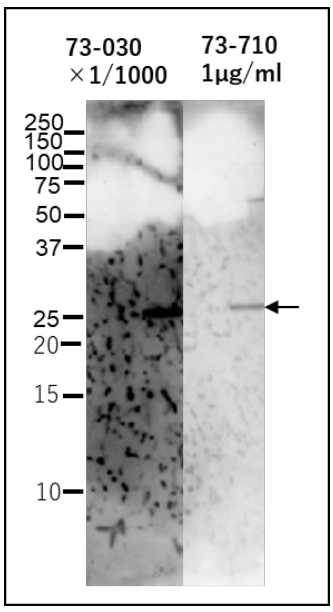


Fig.3. Western blot analysis of CD52 in lysates of mouse testis anti-CD52 antibody. (73-030, 73-710)

Proteins in the mouse testis lysate (10μg) 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 are positions of protein size markers shown in kDa.

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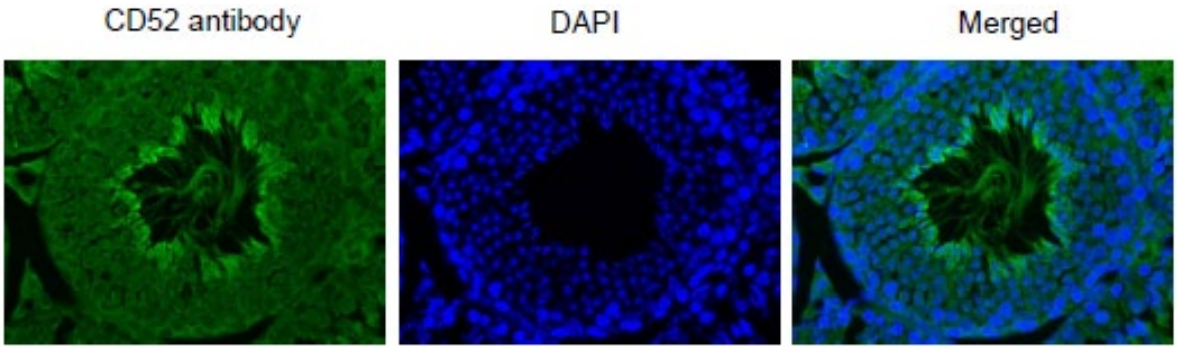


Fig.4. Immunohistochemistry of mouse testis using anti-CD52 antibody.

Formalin-fixed and paraffin-embedded mouse testis

Deparaffinization	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℃ 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)

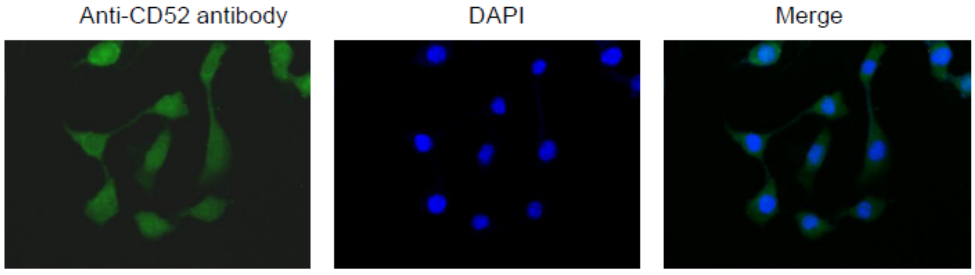


Fig. 5 Immunofluorescence staining of CD52 in NIH3T3 cells with anti-CD52 antibody.

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 withAlex 488 (1/1000 dilution).

Nuclei were stained with DAPI

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