

## Anti-CD52 antibody, rabbit serum

Product code	73-030
Size	100 μl
Storage	Store 4°C for short term For long term storage store at -20°C.
	Aliquot to avoid repeated freezing and thawing.
Concentration	N/A
Buffer	0.1% sodium azide
Purity	Rabbit antiserum
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)
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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

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



Data Images: 73-030 Anti-CD52 antibody, rabbit serum

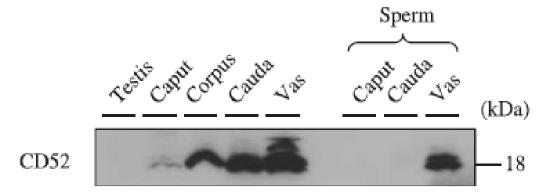


Fig.1 Western blotting analysis of CD52 expression in various tissues with anti-CD52 antibody.

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

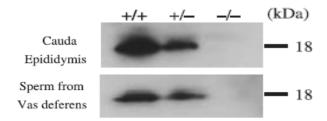


Fig.2. Western blot analysis of CD52 in cauda epididymal lysates and sperm lysates of wild type and CD52 deficient mice.  $Cd52^{+/+}$  (+/+),  $Cd52^{+/-}$  (+/-), and  $Cd52^{-/-}$ (-/-).

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



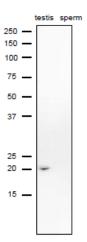


Fig.3. Western blot analysis of CD52 in lysates of mouse testis and sperm with anti-CD52 antibody.

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.

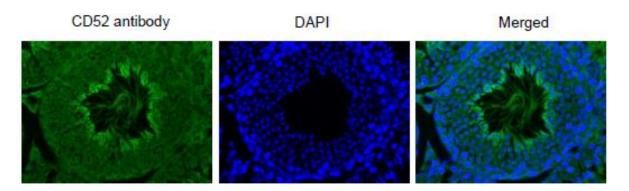


Fig.4. Immunohistochemistry of mouse testis using anti-CD52 antibody.

Formalin-fixed and paraffin-embedded mouse testis

Deparaffinization 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)



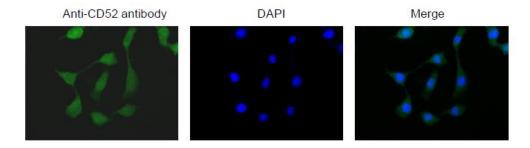


Fig. 5 Immunofluorescence staining of CD52 in NIH3T3 cells with anti-CD52 antibody.

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

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: <u>18782223</u> **WB (mouse)**.