For Research Use Only.
Not for use in diagnostic procedures.

Anti-DBP (Mouse) pAb

CODE No. PM079

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified IgG from rabbit serum
REACTIVITY This antibody reacts with mouse DBP, and will cross-react with overexpressed mouse HLF.
FORMULATION PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATION-CONFIRMED
Western blotting 1:1,000

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	Not tested	Liver nuclear extract, transfectant	Not tested	Not tested
Reactivity		+		

Entrez Gene ID 13170 (Mouse)

For more information, please visit our website at **https://ruo.mbl.co.jp/.**

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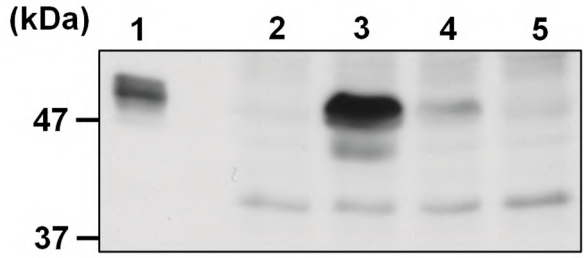
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

Example of protocol for tissue samples

1) Mix 10 μL of Mouse liver nuclear extract with 10 μL of Laemmli's sample buffer.
2) Boil the sample for 5 min. and centrifuge. Load 20 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 300 mA for 1 hr. in a wet transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 1% skimmed milk (in TBS, pH 7.2) for 1 hr. at room temperature.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in TBS, pH 7.2) as suggested in the **APPLICATION** for 2 hr. at room temperature or overnight at 4°C. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane 3 times for 2 min., 5 min. and 10 min. each with 1% skimmed milk (in TBS, pH 7.2).
7) Incubate the membrane with the 1:5,000 anti-IgG (Rabbit) pAb-HRP diluted with 1% skimmed milk (in TBS, pH 7.2) for 2 hr. at room temperature or overnight at 4°C.
8) Wash the membrane 3 times for 2 min., 5 min. and 10 min. each with TBS-T [0.05% Tween-20 in TBS].
9) Wash the membrane 1 time for 2 min. with TBS.
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Mouse liver nuclear extracts)



Western blot analysis of mouse DBP

Lane 1: Flag-tagged mouse DBP
Lane 2: Mouse liver nuclear extracts (ZT6)
Lane 3: Mouse liver nuclear extracts (ZT12)
Lane 4: Mouse liver nuclear extracts (ZT18)
Lane 5: Mouse liver nuclear extracts (ZT24)

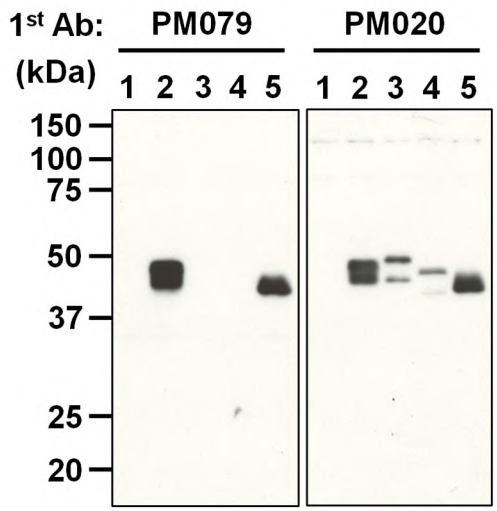
Immunoblotted with Anti-DBP (Mouse) pAb (PM079)

Data was kindly provided by Ms. Rina Nunokawa, Dr. Hikari Yoshitane, and Dr. Yoshitaka Fukada. (Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo)

SDS-PAGE & Western blotting

Example of protocol for cell samples

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL Laemmli's sample buffer.
2) Boil the sample for 5 min. and centrifuge. Load the sample (2 x 10^4 cells) per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with the 1:10,000 anti-IgG (H+L chain) (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



Western blot analysis of mouse DBP

Lane 1: Empty vector/293T
Lane 2: DDDDK-tagged mouse DBP/293T
Lane 3: DDDDK-tagged mouse TEF isoform 1/293T
Lane 4: DDDDK-tagged mouse TEF isoform 2/293T
Lane 5: DDDDK-tagged mouse HLF/293T

Immunoblotted with Anti-DBP (Mouse) pAb (PM079) or Anti-DDDDK-tag pAb (PM020)