

Anti - NEPHRIN

Rabbit clonal antibody

CAT#

DB 017-0.05 (50 µl)
DB 017-0.1 (100 µl)

PRODUCT INFORMATION

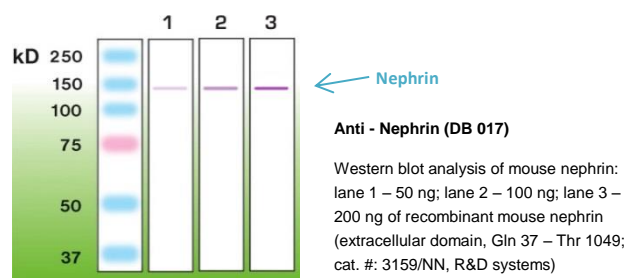
Clone number:	Y17-R	Buffer:	20 mM Tris-HCl, pH 8.0
Uniprot:	Human: O60500; Mouse: Q9QZS7; Rat: Q9R044	Stabilizer:	10 mg/ml BSA
Product description:	Rabbit anti - nephrin clonal IgGs	Preservative:	0.05% Sodium Azide
Basic information:	Major clone of rabbit immunoglobulin corresponding to immunogenic peptide	Storage:	10 µl aliquots at -20°C
Immunogen:	Peptide derived from the centre of the fifth Ig-like domain of human nephrin. Antibody recognizes the epitope located between Cys465 - Cys528.	Handling:	Avoid repeated freezing and thawing
Species reactivity:	Human, mouse, rat - tested	Expiration:	24 months from the shipping date
		Applications:	Western blot, ELISA, Immunoprecipitation
		Dilution range:	Western blotting – 1:5,000 ELISA – 1:50,000-1:100,000

WESTERN BLOT (WB) PROTOCOL - INSTRUCTION MANUAL

Western immunoblotting solutions:

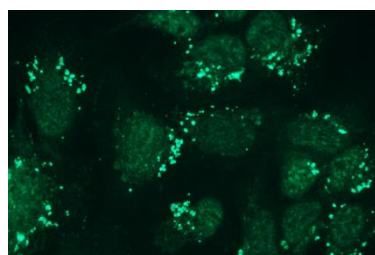
- Wash buffer: 1x Tris Buffered Saline (TBS); 0.1% Triton X-100
- Blocking buffer: 1xTBS; 0.1% Triton X-100; 5% nonfat dry milk

For western blots, incubate the membrane with antibody diluted in blocking buffer for 2 hours at room temperature



IMMUNOCYTOCHEMISTRY (ICC) PROTOCOL - INSTRUCTION MANUAL

1. Coat coverslips with 1% gelatin-coating solution for 2 hours at room temperature (RT); rinse with distilled water, and let to dry overnight. Before plating the cells, wash the coated coverslips briefly with PBS.
2. Fix the cells with 4% paraformaldehyde solution (in PBS, pH 7.2), for 15 min at RT.
3. Wash 2 x 3 min with PBS.
4. Permeabilize the cells with 0.1% Triton X-100 solution (in PBS, pH 7.2) for 5 min on ice.
5. Wash 2 x 3 min with PBS.
6. Incubate the cells in blocking buffer (0.3M glycine in PBS, 2% BSA) for 30 min at RT.
7. Incubate the cells with primary antibody: anti-Nephrin clonal antibody at the dilution of **1:600 - 1:1000** in antibody dilution buffer (PBS, 1% BSA) for 1 hour at RT in humid chamber.
8. Wash 2 x 3 min with PBS.
9. Apply the secondary antibody (*in this case, the goat anti-rabbit IgG-FITC from Jackson ImmunoResearch, cat. # 111-095-003, was used at 1:300 in antibody dilution buffer, and cells were incubated for 1 hour at RT in dark.*)
10. Wash 3 x 3 min with PBS.
11. Rinse once with distilled water.
12. Mount the slide for observation, with a drop of anti-fade mounting medium.



Representative picture of Nephrin expression in HEK293 cells, visualized with clonal rabbit anti-Nephrin monospecific antibody. Primary antibody dilution - 1:700.

PRECAUTIONS

1. Intended for professional In Vitro Diagnostic use in laboratories.
2. Do not use after expiration date stamped on vial label.
3. Avoid contamination of the reagent.
4. Any discrepancies in the recommended procedures stated in the working protocol may affect the final results.
5. The reagent contains sodium azide (NaN₃) which is highly toxic in higher concentrations. The concentration in the reagent (0.05%) is not considered as hazardous.
6. Disposal of waste material must be conducted in accordance with local regulations.
7. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.