

MONOCLONAL ANTIBODY

# Anti-Luciferase mAb

Code No.	Clone	Subclass	Quantity	Concentration
M095-3	2D4	Mouse IgG1	100 $\mu$ L	1 mg/mL

**BACKGROUND:** Luciferase is a 61 kDa luminescent enzyme isolated from *Photinus pyralis* (North American Firefly). The enzyme catalyzes the ATP dependent oxidation of beetle luciferin to produce inorganic phosphate and light. Luciferase is frequently used as a reporter protein to quantify expression levels from promoters in tissue culture and *in vivo*.

**SOURCE:** This antibody was purified from hybridoma (clone 2D4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with recombinant full length luciferase (*Photinus pyralis*).

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L volume of 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.

**REACTIVITY:** This antibody reacts with luciferase (61 kDa) on Western blotting. It does not cross-react with the luciferase derived from *Renilla mullerei*.

## APPLICATIONS:

Western blotting; 1  $\mu$ g/mL

Immunoprecipitation; 5  $\mu$ g

Immunocytochemistry; Not tested

Immunohistochemistry; Not tested

Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

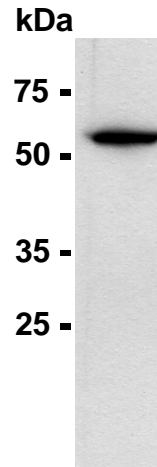
## REFERENCE:

- 1) Xu, G., *et al.*, *Mol. Pharmacol.* **59**, 485-492 (2001)

## INTENDED USE:

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

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



**Western blot analysis of Luciferase expression in pGL3/293T cells using M095-3.**

## PROTOCOLS:

### SDS-PAGE & Western Blotting

- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture'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 hour at room temperature, or overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody to be used will depend on condition.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 6 times).

- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. 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 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

### **Immunoprecipitation**

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggest in the **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.)

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