3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-Luciferase antibody, Mouse monoclonal
clone LUC-1, purified from hybridoma cell culture

Product Number **L2164**

Product Description

Anti-Luciferase antibody, Mouse monoclonal , (mouse IgG1 isotype) is derived from the LUC-1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with firefly (*Photinus pyralis*) luciferase. The isotype is determined by a double diffusion immunoassay using the Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2.

Anti-Luciferase antibody, Mouse monoclonal recognizes recombinant luciferase in transfected eukaryotic (293T) cells. The product is useful in immunoblotting (approx. 60 kDa) and immunocytochemistry (methanol-acetone fixation).

The ability of DNA sequences to function as promoters or enhancers of gene expression may be determined by transient transfection. In such a system, cells are transfected with plasmids encoding a fusion gene, and gene expression is assayed within 48 hours after introduction of the novel DNA. The fusion gene consists of either a promoter binding site or an enhancer sequence that is attached to a reporter gene. The amount of the reporter protein synthesized under the experimental conditions reflects the ability of the promoter or enhancer sequence to direct or promote gene transcription. Several enzymes are commonly used as reporter proteins, including chloramphenicol acetyl transferase (CAT), β-galactosidase, and luciferase. Firefly luciferase from *Photinus pyralis*[1,2] is a widely used reporter enzyme since it catalyzes a bioluminescent reaction that may be directly monitored. The reaction requires the substrate luciferin as well as Mg^{+2} and ATP. Mixing these reagents with the cell extract containing luciferase, elicits a flash of light that decays rapidly and can be detected by means of a luminometer. The total light emission is proportional to the luciferase activity of the sample. The luciferase assay is rapid, sensitive, and, unlike the CAT assay, does not require a radioactive substrate. However, the luciferase assay requires access to a luminometer and may lack reproducibility between samples, largely due to the rapid kinetics of the light emission.

Anti-Luciferase provides a stable, inexpensive alternative detection assay for luciferase. Since this assay directly detects the luciferase protein, it has the advantage that it is not dependent upon either luciferase activity nor the measurement of rapid reaction kinetics. Moreover, antibodies can detect the luciferase enzyme expression *in situ*, providing a means to study the cellular localization of the signal sequence activation. Several different detection systems are available depending on the secondary antibody employed.

Reagents

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: ~2 mg/ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in a frost-free freezer, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 2-4 μg/ml is determined using a whole extract of transfected 293T (human embryonal kidney) cells expressing luciferase.

Immunocytochemistry: a working concentration of 20-40 μg/ml is determined using methanol-acetone fixation of transfected 293T (human embryonal kidney) cells expressing luciferase.

Note: In order to obtain the best results using different techniques and preparations we recommend determining the optimal working concentration by titration.

Procedures

Procedure for Immunoblotting

Note: All incubations should be performed at room temperature.

1. Separate luciferase protein from the sample lysate using a standard sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5-20 μg total lysate protein per lane. The amount of lysate to be loaded per lane depends on the level of protein expression; thus the optimum loading may vary between preparations.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk in phosphate buffered saline (PBS) for 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (Product No. P3563).
   Optional: Block with PBS containing 1% BSA for 10 minutes, followed by draining. This procedure may minimize non-specific adsorption of the antibodies.
5. Incubate the membrane with Monoclonal Anti-Luciferase antibody as the primary antibody for 120 minutes with agitation in PBS containing 0.05% TWEEN 20.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane with Anti Mouse IgG-Alkaline Phosphatase, Product No. A 2179 or A1682, as the secondary antibody, at the recommended concentration in PBS containing 0.05% TWEEN 20, with agitation for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
9. Incubate the membrane in an Alkaline phosphatase substrate solution.

Procedure for Indirect Immunofluorescent Staining of Cultured Cells

Note: All incubations, except steps 1 and 3, should be performed at room temperature.

1. Grow transfected cultured cells expressing luciferase protein on sterile coverslips at 37 °C.
2. Wash the coverslips briefly in phosphate buffered saline (PBS).
3. Fix the cells with −20 °C methanol (10 minutes) and then with −20 °C acetone (1 minute).
4. Wash the coverslips twice in PBS (5 minutes each wash).
   Optional: Block with PBS containing 1% BSA for 10 minutes, followed by draining. This procedure may minimize non-specific adsorption of the antibodies.
5. Incubate the coverslips cell-side-up with Monoclonal Anti-Luciferase antibody in PBS, for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate the coverslips cell-side-up with Anti-Mouse IgG-FITC, Product No. F2653 or F5262, as the secondary antibody at the recommended dilution in PBS containing 1% BSA, for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of aqueous mounting medium on the coverslip, and invert it carefully on a glass slide. Avoid air bubbles.
10. Examine using a fluorescence microscope with the appropriate filters.

References

1. de Wet, J.R., et al., *Proc. Natl. Acad. Sci. USA*, **82**, 7870-7873 (1985).
2. de Wet, J.R., et al., *Mol. Cell. Biol.*, 7, 725-737 (1987).

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