Certificate of Analysis

Anti-NanoLuc® Monoclonal Antibody:

Part#:	Size
N700A	100µg

Description: Anti-NanoLuc® Monoclonal Antibody (Cat.# N7000) is a protein A/G affinity-purified mouse monoclonal antibody that is used to detect NanoLuc® luciferase or NanoLuc® fusion proteins via Western blotting. When Anti-NanoLuc® Monoclonal Antibody was used in Western blots, no cross-reactivity with the LgBiT subunit of NanoBiT® technology was observed.

Dilution: We recommend a concentration of 1µg/ml as a starting point for protocol optimization.

Expiration Date: See product label for expiration date.

Form: Lyophilized.

Isotype: Mouse IgG2a.

Reconstitution: Reconstitute to 0.5mg/ml using sterile PBS.

Storage Conditions: Store at –30°C to –10°C. The reconstituted material can be stored for 1 month at +2°C to +10°C or for 6 months below –10°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

Usage Notes:

1. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.
2. The sensitivity of the Anti-NanoLuc® Monoclonal Antibody may be insufficient to detect NanoLuc® Luciferase or NanoLuc® Luciferase fusions expressed at very low levels, e.g., transient expression via CMV-based expression constructs diluted with Transfection Carrier DNA or expression via weak promoters like HSV-TK.

Quality Control Assays

This product passes the following Quality Control specifications:

Purity: Anti-NanoLuc® Monoclonal Antibody shows ≤10% aggregation by size exclusion chromatography.

Bioburden: Bioburden testing was performed using direct plate and broth dilution methods.

Usage Information on Back

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIN7000

Printed 2/20

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Promega

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Usage Information

Example Protocol

The following protocol was used to generate the results shown in the gel image. This protocol can serve as a starting point for protocol optimization. Alternative protocols/reagents are also viable.

Materials to Be Supplied by the User

- blotted membrane
- tray for incubating and washing membrane
- SuperBlock™ (TBS) Blocking Buffer (Thermo Scientific Cat.# 37535)
- Tris-buffered saline containing 0.1% v/v Tween 20 (1X TBST)
- Anti-Mouse IgG (H+L), HRP Conjugate (Cat.# W4021)
- orbital shaker or rocking platform
- ECL Western Blotting Substrate (Cat.# W1015)
- CCD imager or X-ray film

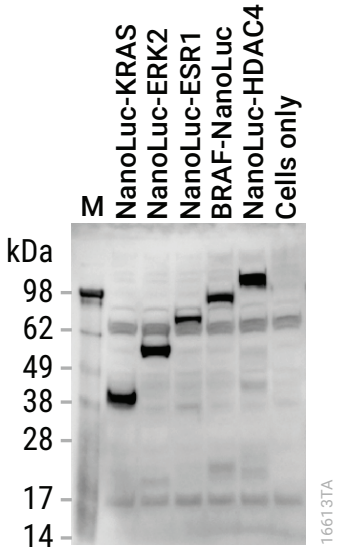
1. After protein transfer, remove membrane from the transfer apparatus, and block nonspecific sites using SuperBlock™ (TBS) Blocking Buffer for 1 hour at room temperature with gentle shaking/rocking.

   Note: Mix SuperBlock™ (TBS) Blocking Buffer well prior to use.

2. Dilute Anti-NanoLuc® Monoclonal Antibody to 1µg/ml using SuperBlock™ (TBS) Blocking Buffer. Remove blocking solution and add Anti-NanoLuc® Monoclonal Antibody solution. Incubate at 4°C overnight with gentle rocking/shaking.
3. Wash 3 times, five minutes per wash, using 1X TBST.
4. Dilute Anti-Mouse IgG (H+L), HRP Conjugate 1:2,500 using SuperBlock™ (TBS) Blocking Solution. Incubate membrane for 1 hour at room temperature with gentle shaking/rocking.
5. Wash 3 times, five minutes per wash, using 1X TBST.
6. Prepare the ECL Western Blotting Substrate working solution by mixing equal parts of the Peroxide Solution and the Luminol Enhancer Solution. Mix just enough substrate to cover the membrane (e.g., 6–7ml per 10cm × 5cm membrane).

   Note: For best results, use the prepared substrate working solution immediately after mixing. The solution is stable for up to 1 hour at room temperature.

7. Incubate the membrane for 1 minute at room temperature.
8. Remove the membrane from solution, blot excess liquid with an absorbent towel, and place in a plastic sheet protector or clear plastic wrap.
9. Image using a CCD camera or expose to X-ray film.



Detection of NanoLuc® fusion proteins expressed in HEK293 cells. HEK293 cells were transfected with CMV-based expression constructs encoding NanoLuc® fusion proteins or Transfection Carrier DNA (Cat.# E4881) using 1µg/ml plasmid DNA per T75 flask (10ml) and FuGENE® HD Transfection Reagent (Cat.# E2311) at a 3:1 lipid:DNA ratio. After 24 hours, cells were washed with PBS and lysed using ice-cold Mammalian Lysis Buffer (Cat.# G9381) supplemented with Protease Inhibitor Cocktail (Sigma Cat.# P8340). Lysates were sonicated and cell debris was pelleted in a microcentrifuge at 12,000 rpm for 10 minutes at 4°C. Total protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Cat.# 23227) following the manufacturer's recommendations. Lysates were resolved using a Bolt 4–12%, Bis-Tris Plus gel (Thermo Fisher Scientific Cat.# NW04122) using MES running buffer (15µg of total protein per lane) and transferred to an iBlot™ 2 Transfer Stack, PVDF (Lane M; Thermo Fisher Scientific Cat.# IB24001) using an iBlot™ 2 Transfer Device. SeeBlue™ Plus2 Pre-Stained Protein Standard (Thermo Fisher Scientific Cat.# LC5925) was used as a molecular weight standard. The protocol listed above was used for Western blot detection with the Anti-NanoLuc® Monoclonal Antibody, and visualized with an ImageQuant LAS4000 imaging system (GE Healthcare) and a 10-second integration time (chemiluminescence, high sensitivity). The actual mobility approximated the expected mobility for each of the NanoLuc® fusion proteins. Background bands are evident in all lanes, including HEK293 cell lysate, which lacks expression of a NanoLuc® fusion protein.

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Printed in USA 2/20.