RayBio® m7G (N7-methylguanosine)

Dot Blot Kit

Catalog #: DB-m7G

User Manual
Last revised April 15th, 2024

RayBiotech
Empowering your proteomics

ISO 13485 Certified

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Please read the entire manual carefully before starting your experiment

I. Introduction

N7-methylguanosine (m7G), is a posttranscriptional modification of RNA that plays an essential role in RNA processing, metabolism, and function. m7G is the most ubiquitous mRNA cap modification and is also present in internal mRNA, microRNA, tRNA, and rRNA. m7G is positively charged and produced by the methyltransferase-like 1 (METTL1) and WD repeat domain 4 (WDR4) complex. Emerging evidence suggests that the METTL1/WDR4 complex plays a role in brain development and disease pathogenesis. m7G has also been implicated in many types of tumorigeneses, including head, neck, lung, liver, colon, bladder, and teratoma. Investigations into m7G have found that this modification was crucial for tumor chemoresistance, vasculogenesis and prognosis. Determining the global changes in m7G levels is necessary to understand both the physiological and pathological states.

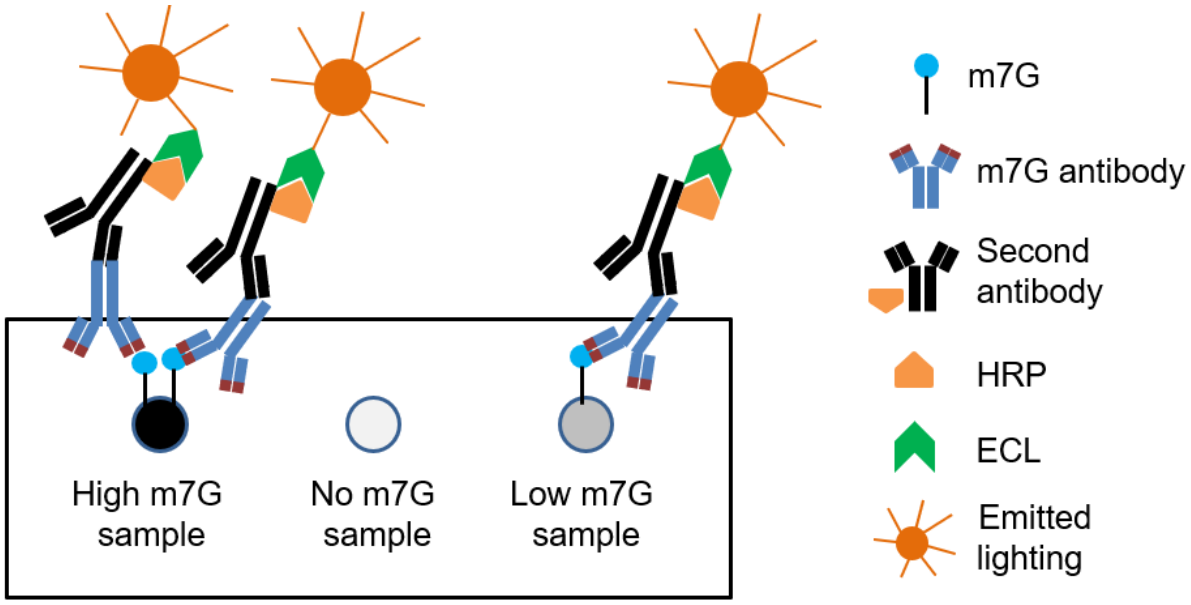
The global m7G level can be measured by dot blot or ELISA analysis using m7G-specific antibodies, mass spectrometry following chromatographic separation, or a high-throughput sequencing platform. Here, we describe an m7G dot blot kit that is a reliable, simple, and rapid method of quantifying the global m7G levels in RNA, urine, serum, plasma, or other nucleic samples collected from humans, rats, and mice.

II. General Description

The RayBio® m7G dot blot Kit is an in vitro semi-quantitative assay for detecting m7G. The sample including RNA is directly spotted onto a nitrocellulose (NC) or nylon membrane followed by incubation with the primary and secondary antibodies. Compared with traditional blotting techniques, our optimized dot blot is a powerful tool for analyzing m7G. This kit is highly sensitive and specific**,** requires minimal sample preparation, no specialty equipment, and can be completed within 3-4 hours, making it a cost-effective and efficient option. Moreover, dot blotting presents a convenient method for conducting high-throughput screening in large-scale studies involving a substantial volume of clinical and experimental samples. It is important to note that dot blotting is a semi-quantitative technique, and quantitative measurements of RNA modifications may require other techniques such as RNA sequencing or mass spectrometry.

In this assay, the sample containing denatured RNA is directly spotted onto a nitrocellulose (NC) membrane. Then, the RNA sample is crosslinked to the membrane using UV light, blocked for 1 hour, and incubated with the anti-m7G antibody for another hour. After a wash step, any bound m7G antibody then interacts with an HRP-secondary antibody for 1 hour, which catalyzes a color development reaction with the ECL substrate.

III. How It Works



Graphical representation of m7G quantification by bot blot assay

IV. Storage

The entire kit may be stored at -20°C to -80°C for up to 12 months from the date of shipment. For extended storage, it is recommended to store it at -80°C. **Avoid repeated freeze-thaw cycles.** For prepared reagent storage, see table below.

V. Reagents

Component	**Size / Description**	**Storage / Stability After Preparation**
m7G Antibody (1000X)	1 vial (10 µl)	1 month at 4°C
Anti-Rabbit IgG-HRP antibody (1000X)	1 vial (10 µl)	1 month at 4°C
Detection Buffer C	1 bottle (10 ml)	1 month at 4°C
Detection Buffer D	1 bottle (10 ml)	1 month at 4°C
Wash Buffer (20X)	1 bottle (30 ml)	1 week at 4°C
Blocking Buffer (4X)	1 bottle (30 ml)	1 month at 4°C
MB staining buffer	1 bottle (30 ml)	Room temperature
NC Membrane	4 sheets (6 x 8cm)	Room temperature
Plastic sheet	8 sheets (7 x 11cm)	Room temperature
Black incubation Box	1 (8.9 cm x 6.5 cm x 2.8 cm)	Room temperature

VI. Additional Materials Required

1. RNase-free tubes
2. Heat blocker
3. 10 cm plastic petri dish
4. UV cross-linker
5. Chemiluminescent Imaging system

VII. Assay Procedure

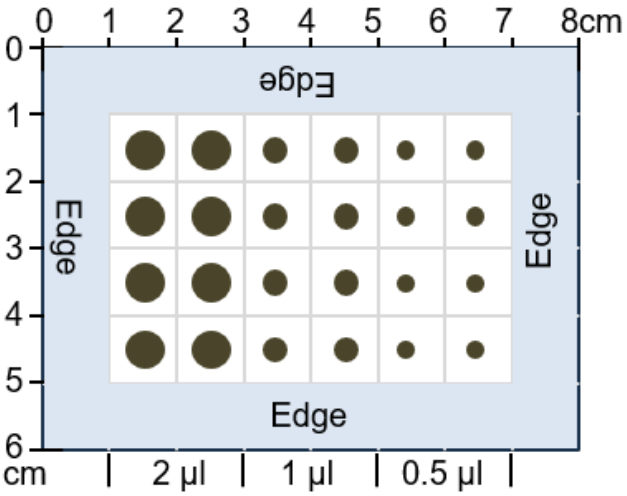
1. Sample preparation:

I. RNA Samples: Extract RNA from cell or tissue samples with TRIZOL reagent (or kit) and formulate a proper final concentration between 200-2000 ng/μL. Dissolve purified RNA/DNA in nuclease-free water at 100-1000 ng/dot with 0.5-2 μl. For optimal quantification, the input RNA amount should be 300 ng/dot in 1 μl.
II. Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with EDTA, heparin or citrate and centrifuge at 5000 g for 10 minutes at room temperature. Remove the plasma layer and avoid disturbing the white buffy layer. Dilute plasma sample with nuclease-free water at 1:3 for dot blot assay.
III. Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 5000 g for 10 minutes at room temperature. Remove the yellow serum supernatant without disturbing the white buffy layer. Dilute serum sample with nuclease-free water at 1:3 for dot blot assay.

2. Incubate the diluted samples at 95°C in a heat block for 5 minutes to remove any RNA secondary structure.

3. Chill the tubes on ice for 5 minutes rapidly after denaturation to prevent the re-formation of secondary structures of RNA.

4. Cut the NC membrane to an appropriate size. Mark the grid on the membrane lightly with a pencil to guide sample loading. This is an example of a dot blot.



5. Mix the sample by pipetting it up and down. Using a narrow-mouth pipette tip, dispense 1-2 µL of the sample onto the membrane in duplicates. The duplicate spots are necessary for running the m7G dot blot as well as the methylene blue-staining which serves as an internal control.

NOTE: Avoid touching the membrane with the pipette tip. Let the pipetted RNA droplets diffuse onto the membrane via surface tension. Change tips after each loading, even between the same sample.

6. Air dry at room temperature for 5 minutes.

7. Using the UV crosslinker set to the Autocross link mode (1,200 microjoules [x100]; 25-50 sec), crosslink RNA to the membrane twice.

8. Wash the membrane in 10 ml of wash buffer in a clean washing tray, which does not need to be RNase-free, for 5 minutes at room temperature with gentle shaking to wash off the unbound RNA.

9. Cut off one line of the spotted sample replicates from the membrane to use for methylene blue staining. Transfer the membrane to a petri dish containing 10 mL methylene blue staining buffer for 10 minutes with gentle shaking.

10. Wash the membrane with dH_2O for 10 minutes until the background is clean. Image the methylene blue-stained membrane with your imaging system.

11. Incubate the second half of the membrane with 1x blocking buffer on a rotating shaker for 1 hour at room temperature.

12. Decant the blocking solution and rinse the membrane with 1x Washing buffer.

13. Incubate the membrane with anti-m7G antibody (1:1,000 dilution) in antibody dilution buffer for 1 hour at room temperature or overnight at 4 °C with gentle shaking.

14. Discard the primary antibody and wash with 1x Washing buffer for 5 – 10 minutes on a rotating shaker. Repeat this wash step 2 additional times.

15. After washing, incubate the membrane with the diluted 1x secondary antibody for 1 hour at room temperature with gentle shaking.

16. Discard the secondary antibody and wash with 1x Washing buffer for 5 – 10 minutes on a rotating shaker. Repeat this wash step 2 additional times.

17. Using a pipette, mix equal volumes (1:1) of Detection Buffer C and Detection Buffer D in a single clean tube.

18. Place the membrane on top of the provided plastic sheet and gently pipette 500 µl of the Detection Buffer mixture onto the membranes. Incubate for 2 minutes at room temperature. Place another plastic sheet on top of the membranes to "sandwich" the membrane. Then, gently roll the flexible plastic sheet across the surface to the opposite end to smooth out any air bubbles.

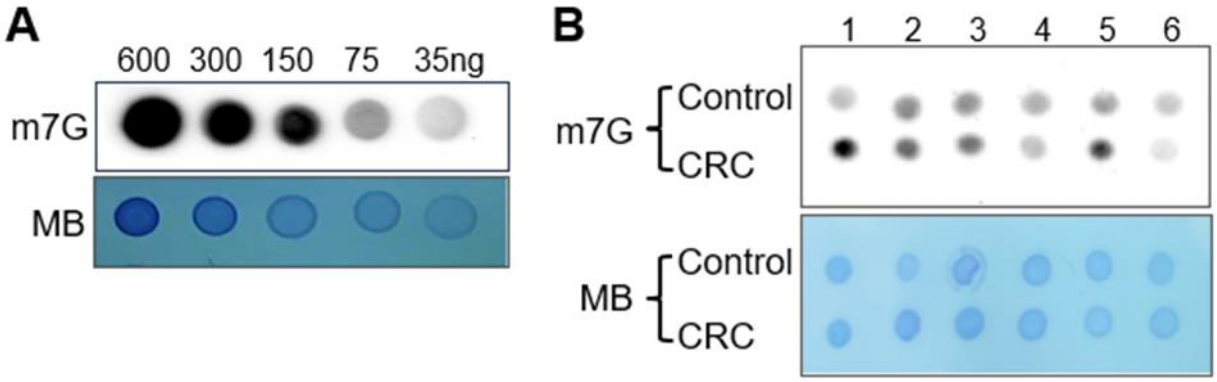
NOTE: Exposure should ideally start within 5 minutes after finishing Step 18 and be completed within 10-15 minutes as chemiluminescence signals will fade over time.

19. Transfer the sandwiched membranes to the chemiluminescence imaging system such as a CCD camera (recommended) and expose.

NOTE: Optimal exposure times will vary so performing multiple exposure times is strongly recommended.

20. To obtain m7G levels from the chemiluminescent images, Image J software (or similar image processing and analysis software) can be used.

VIII. Typical Results



Representative image of m7G-specific dot blot assay of RNA and plasma samples. Total RNA was isolated from the human lung cancer cell line of A431 and quantified by nanodrop, respectively (A). Plasma isolated from colorectal cancer (CRC) and normal health controls (B). m7G dot blot assay followed this protocol.

IX. Troubleshooting Guide

PROBLEM	CAUSE	RECOMMENDATION
No signals (not even the positive controls spots)	Chemiluminescent imager is not working properly	Contact image manufacturer
	Too Short Exposure	Ensure sufficient incubation time and change sample incubation step to overnight
	Degradation of components due to improper storage	Store entire kit at ≤ - 20°C. Do not use the kit after the expiration date. See storage guidelines.
	Improper preparation or dilution of the antibodies	Centrifuge vial briefly before use, mix well, and do not dilute more than 1000-fold
	Waiting too long before exposing	The entire detection process should be completed in 10-15 minutes
	Low sample protein levels	Decrease sample dilution, concentrate samples, or load more protein initially
Uneven signals and/or background	Bubbles present on or below membrane	Don’t rock/rotate the tray too vigorously or pipette the sample or reagent with excessive force
	Insufficient sample or reagent volume	Load enough sample and reagent to completely cover the membrane
	Insufficient mixing of reagents	Gently mix all reagents before loading onto the membrane, especially the HRP-second antibody
	Rocking/Rotating on an uneven surface while incubating	Rock/rotate on a flat surface or the sample or reagent can “pool” to one side
High background signals or all spots visible	Too much HRP-second Antibody Cocktail	Prepare these signal enhancing components precisely as instructed
	Membranes dried out	Do not let the membranes dry out during the experiment. Cover the incubation tray with the lid to minimize evaporation
	Too High of Sample Protein Concentration	Increase dilution of the sample or load less protein
	Exposed Too Long	Decrease exposure time
	Insufficient Washing	Ensure all the wash steps are carried out and the wash buffer is removed completely after each wash step
	Non-specific binding	Ensure the blocking buffer is stored and used properly.

RayBio® ELISA Kits

Over 3,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

This product is for research use only.