m7G (N7-methylguanosine) Competitive ELISA kit

Catalog #: EIA-m7G

User Manual
Last revised May 6th, 2024

RayBiotech
Empowering your proteomics

ISO 13485 Certified

Table of Contents

Please read the entire manual carefully before starting your experiment

I. Introduction

Currently, more than 170 modifications have been identified on RNA, which affect RNA metabolism and are involved in many different biological processes. As one of the prevalent posttranscriptional modifications of RNA, N7-methylguanosine (m7G) plays essential roles in RNA processing, metabolism, and function. m7G is the most ubiquitous mRNA cap modification and is also present in internal mRNA, microRNA (miRNA), 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 promoted or inhibited the processes of brain development and diseases, as well as many kinds of tumor, including head and neck, lung, liver, colon, bladder cancer, and teratoma. Additionally, m7G is also crucial for tumor chemoresistance, vasculogenesis and prognosis. Therefore, m7G modification can be used as biomarkers or potential intervention targets, providing new possibilities for early diagnosis and treatment of tumors.

The RayBio® m7G ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of m7G in urine, serum, plasma, and RNA samples collected from human, rat or mouse. The quantity of m7G is determined by a known m7G standard curve. The m7G kit has a high sensitivity of approximately 0.164 ng/ml. The performance was also verified to exhibit good characteristics in terms of linearity, stability, precision, and spiking & recovery. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

II. General Description

The RayBio® m7G Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting m7G based on the competitive enzyme immunoassay principle.

In this assay, the samples and standards are added to a microplate pre-coated with m7G. In the plate wells, the endogenous m7G or the standards compete with the pre-coated m7G for binding to the anti-m7G antibody. After a wash step, any bound m7G antibody then interacts with horseradish peroxidase (HRP)-secondary antibody, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of bound m7G antibody and inversely proportional to the amount of endogenous m7G in the standard or samples. A standard curve of known concentration of m7G can be established and the concentration of m7G in the samples can be calculated accordingly.

III. How It Works
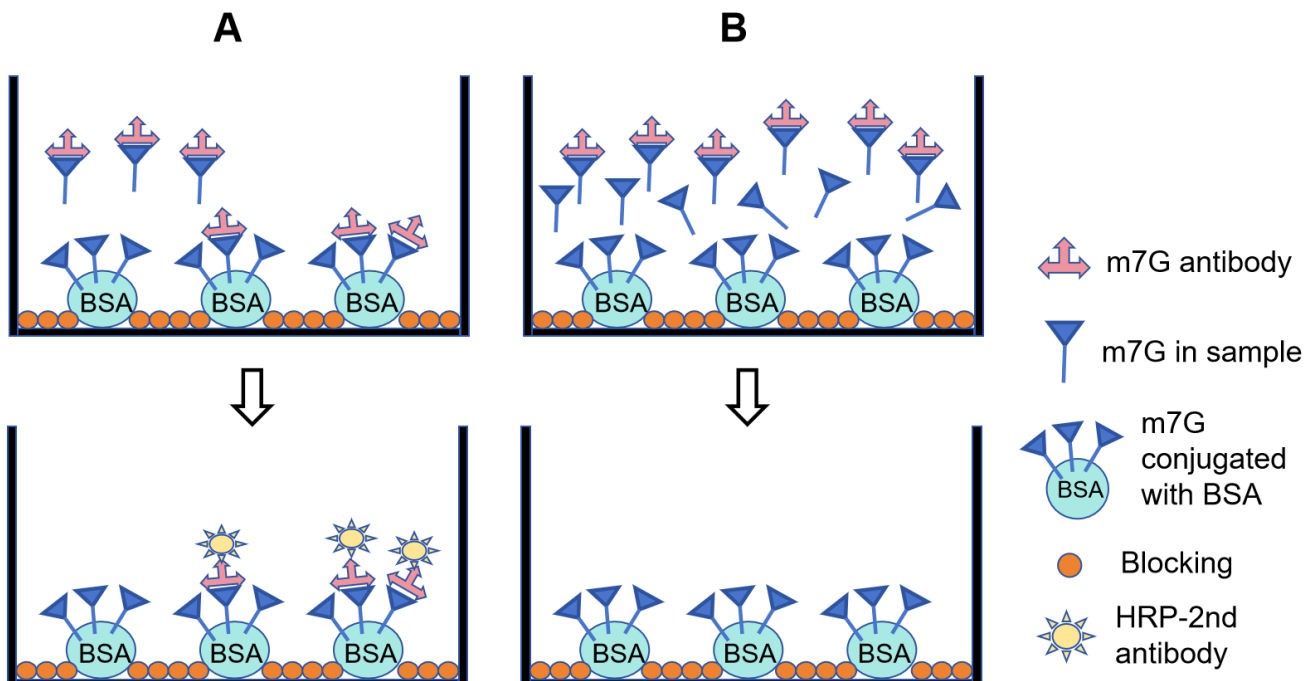
A. Low to moderate level of endogenous m7G in sample. Anti-m7G binds to coated m7G due to low competition and is then detected by HRP-conjugated secondary antibody. **B.** High level of endogenous m7G in sample. Anti-m7G is bound by excess free m7G and washed off the plate.

IV. Storage

The entire kit may be stored at -20°C to -80°C for up to 6 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
EIA-m7G Microplate	96 wells (12 strips x 8 wells) coated with m7G.	1 month at 4°C*
Assay Diluent B (5X)	15 ml concentrated buffer. Diluent for standards, samples, and antibodies.	1 month at 4°C
Standard m7G	2 vials. 1 vial is enough to run in three times.	1 week at 4°C
Positive Control	2 vials of Lyophilized Positive Control. 1 vial is enough to run in three times.	1 month at 4°C
Anti-m7G Antibody (1000X)	10 µl concentrated anti-m7G Antibody.	1 month at 20°C
HRP-conjugated secondary antibody (5000X)	5 µl concentrated antibody.	Do not store and reuse
Wash Buffer (20X)	25 ml of 20X concentrated solution.	1 month at 4°C
TMB One-Step Substrate Reagent	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	1 month at 4°C
Stop Solution	12 ml of 0.2 M sulfuric acid.	12 months at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

VI. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1-liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. GraphPad Prism or SigmaPlot software (or other software which can perform four-parameter logistic regression models).
8. Tubes to prepare standard or sample dilutions.
9. Orbital shaker.
10. Aluminum foil.
11. Plastic wrap.

VII. Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

A. Preparation of Plate and Anti-m7G Antibody

1. Equilibrate plate to room temperature before opening the sealed pouch.

2. Label removable 8-well strips as appropriate for your experiment.

3. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water.

4. Briefly centrifuge the anti-m7G antibody vial and dilute 1000-fold with 1X Assay Diluent B for working solution.

B. Preparation of Standards

5. Label 8 microtubes with the following concentrations: S1 (4000 ng/ml), S2 (400 ng/ml), S3 (40 ng/ml), S4 (4 ng/ml), S5 (0.4 ng/ml), S6 (0.04 ng/ml), S7 (0.004 ng/ml) and S8 (0 ng/ml). Pipette 180 µl of 1x Assay Diluent B into S2-S8.

6. Briefly centrifuge the vial of m7G Standard. Reconstitute with 200 µl of 1x Assay Diluent B. Mix thoroughly. This solution serves as S1.

7. To make the S2 (400 ng/ml) standard, pipette 20 µl of the S1 standard into the tube labeled S2 containing 180 µl of 1x Assay Diluent B. Mix thoroughly.

8. Repeat this step with successive concentration, preparing a dilution series as shown in the illustration below. Each time, add 20 µl of the prior concentration until the S7 is reached. Mix each tube thoroughly before the next transfer. 1x Assay Diluent B serves as the blank (S8, 0 ng/ml).
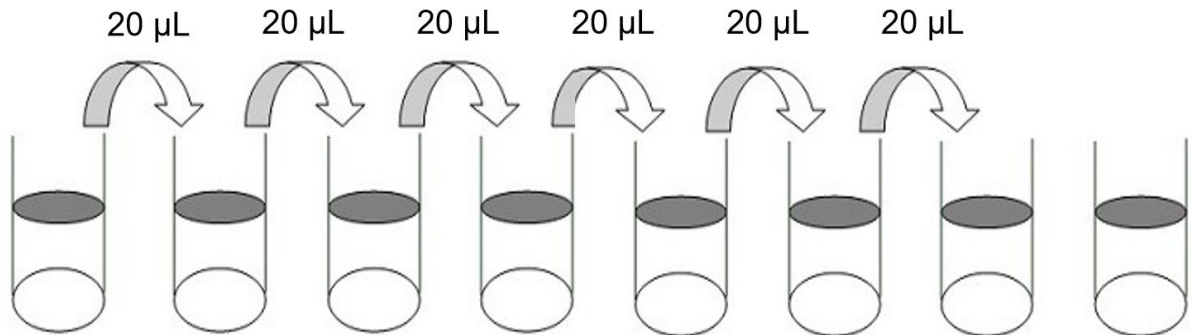
	S1	S2	S3	S4	S5	S6	S7	S8
Diluent Volume	200 µl	180 µl	180 µl	180 µl	180 µl	180 µl	180 µl	180 µl
Conc.	4000 ng/ml	400 ng/ml	40 ng/ml	4 ng/ml	0.4 ng/ml	0.04 ng/ml	0.004 ng/ml	0 ng/ml

C. Positive Control Preparation

9. Briefly centrifuge the Positive Control vial and reconstitute with 200 µl of 1x Assay Diluent B.

Positive Control is a mouse serum sample that serves to verify that the kit components are functioning. The resulting OD is not used to calculate final concentrations of samples. If no positive competition is observed please contact RayBiotech Technical Support. The Positive Control may be diluted further if desired.

D. Sample Preparation

10. Urine, serum, and plasma samples from humans, rats, and mice can be detected. Use immediately or aliquot and store at -20 ºC until use. Avoid repeated freeze-thaws. Dilute your sample with the 1x Assay Diluent B at 1:4, for example 10 µl of sample add into 40 µl 1x Assay Diluent B.

I. Urine: Urine should be aseptically collected from the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge samples at 5000 g for 10 minutes, or filter through 0.45 µm filter, prior to use in the assay.

II. 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 x g for 10 minutes at room temperature. Remove the yellow serum supernatant without disturbing the white buffy layer.

III. 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.

IV. Cell or Tissue RNA Sample:
1. Dissolve total RNA, mRNA or small RNA from cell or tissue samples in nuclease free water at 0.1-2 mg/ml.
2. Remove any RNA secondary structure by incubating the sample at 95ºC for 5 minutes and rapidly chilling on ice.
3. Centrifuge the reaction mixture for 5 minutes at 6000 x g at 4 ºC and collect the supernatant (1-5 µg of RNA sample) for EIA assay.

If you have any questions regarding the recommended dilutions, you may contact technical support at 888-494-8555 or techsupport@raybiotech.com.

E. Preparation of Wash Buffer and HRP

11. If Wash Buffer contains visible crystals, warm them to room temperature and mix gently until dissolved.

12. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

13. Briefly centrifuge the HRP-Secondary Antibody vial before use. Dilute the HRP-Secondary Antibody concentrate 5000-fold with 1X Assay Diluent B.

VIII. Assay Procedure

1. Remove the appropriate number of microtiter wells of m7G coated microplate from foil pouch and place them into the well holder. Return any unused wells to the foil pouch with desiccant pack, reseal along entire edge, and store at -20 ⁰C.

2. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

3. Add 50 µl of each standard (S1-S8), Positive Control and sample to appropriate wells. Incubate at room temperature for 10 minutes on an orbital shaker.

4. Add 50 µL of the diluted Anti-m7G Antibody to each well. Cover wells and incubate at room temperature for 1 hour on an orbital shaker.

5. Discard the solution and wash wells 5 times with 1X Wash Solution Buffer (200-300 µl each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 100 µl of prepared HRP-Secondary antibody solution (see Reagent Preparation step 13) to each well. Incubate for 1 hour at room temperature with gentle shaking.

7. Discard the solution and wash 5 times as directed in Step 5.

8. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 2-10 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

9. Add 100 µl of Stop Solution to each well. Read at 450 nm immediately. Results should be read immediately (color will fade over time).

   Note: Reliable standard curves are obtained when OD values do not exceed 2.5 units for the blanks (S8).

IX. Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

2. Add 50 µl standards or samples to appropriate well. Incubate for 10 minutes at room temperature.

3. Add 50 µl anti-m7G to appropriate well. Incubate 1 hour at room temperature. And wash 5 times.

4. Add 100 µl prepared HRP-Secondary antibody solution. Incubate 1 hour at room temperature. And wash 5 times.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 2-10 minutes at room temperature.

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

X. Calculation of Results

Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using GraphPad Prism or Sigma Plot software (or other software which can perform four-parameter logistic regression models). If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The measurement range is 0.164 – 1000 ng/ml. Any sample reading lower than the highest standard should be diluted with dilution buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the m7G concentration.

Percentage absorbance = B/B0 X 100%, where
B = OD of sample or standard
B0 = OD of Blank (S8, or zero standard)

A. Typical Data
These standard curves are for demonstration only. A standard curve must be run with each assay.
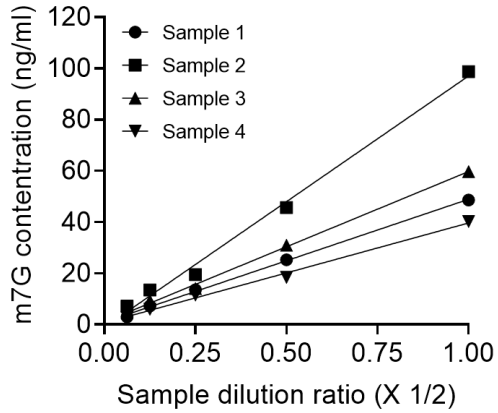

m7G standards (ng/ml)

m7G standards (ng/ml)
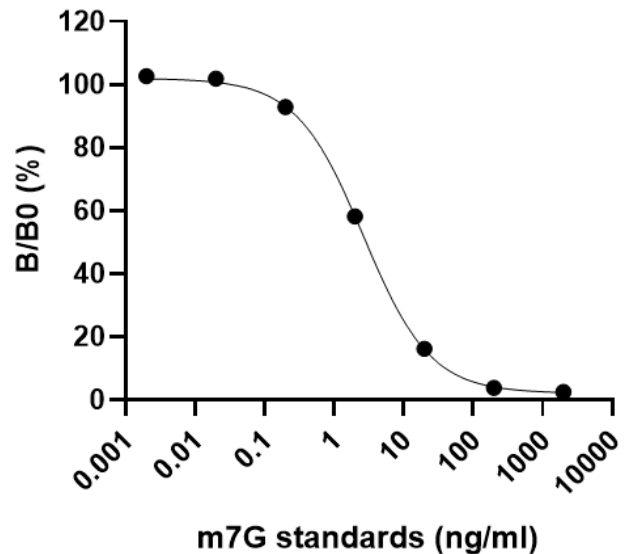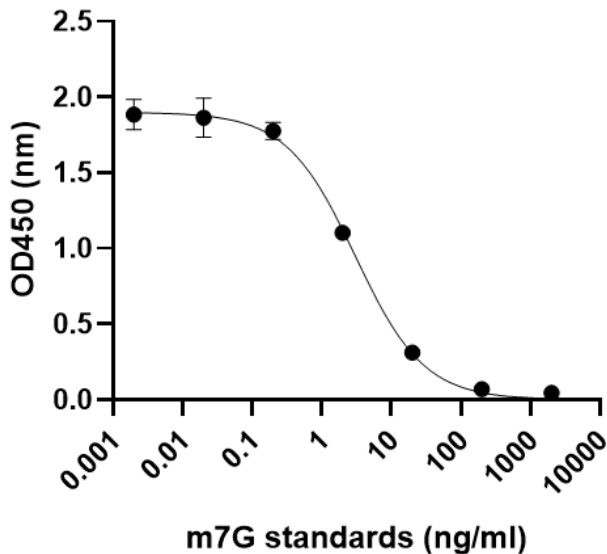
B. Sensitivity
The limit of detection (LOT, Blank-3SD) is 0.164 ng/ml.

C. Linearity

Human serum, plasma and urine samples were serially diluted from 2~32-fold (n=3). The recovery ranges from 79.563 to 126.743.

	Dilution	Sample 1	Sample 2	Sample 3	Sample 4
Observed (ng/ml)	2	48.593	98.725	59.682	40.198
	4	25.248	45.714	30.985	18.398
	8	13.535	19.473	14.067	11.505
	16	7.229	13.487	9.279	5.987
	32	2.927	7.187	4.688	2.666
Expected (ng/ml)	2	48.593	98.725	59.682	40.198
	4	24.296	49.362	29.841	20.099
	8	12.148	24.681	14.920	10.049
	16	6.074	12.341	7.460	5.025
	32	3.037	6.170	3.730	2.512
Recovery (%)	2	100.000	100.000	100.000	100.000
	4	96.230	107.980	96.306	109.247
	8	89.752	126.743	106.065	87.345
	16	84.026	91.502	80.402	83.931
	32	103.763	85.855	79.563	94.231

C. Precision

Intra-assay (Within-Run, n=5) CV%=3.247-8.084. Three cases of human urine, plasm and serum samples were tested in 8 separate assays, respectively.

Repeat time	Urine 1 (ng/ml)	Urine 2 (ng/ml)	Urine 3 (ng/ml)	Plasma 1 (ng/ml)	Plasma 2 (ng/ml)	Plasma 3 (ng/ml)	Serum 1 (ng/ml)	Serum 2 (ng/ml)	Serum 3 (ng/ml)
1	47.255	126.659	60.812	32.555	29.045	72.168	33.462	56.574	47.697
2	46.992	130.219	59.833	32.444	29.045	71.225	32.499	52.532	48.415
3	46.818	129.014	58.172	33.290	29.242	67.341	34.696	47.519	47.079
4	53.765	151.908	63.625	35.180	30.201	80.434	39.352	48.963	49.801
5	55.145	147.799	63.758	34.997	29.144	71.225	38.731	51.435	51.633
6	49.519	138.763	61.185	34.636	29.491	75.111	35.983	55.579	46.473
7	48.688	132.271	60.197	33.347	26.963	74.276	33.347	46.992	50.179
8	49.240	138.763	59.113	32.499	28.461	78.598	31.730	49.240	49.519
Mean	49.678	136.924	60.837	33.619	28.949	73.797	34.975	51.104	48.849
SD	3.141	9.135	1.997	1.154	0.940	4.253	2.827	3.584	1.739
CV%	6.322	6.671	3.283	3.432	**3.247**	5.764	**8.084**	7.013	3.560

Inter-assay (Run-to-Run, n=4) CV%=4.911-11.124. Three cases of human urine and plasm samples were tested in 4 separate assays.

| Repeat time	Urine 1 (ng/ml)	Urine 2 (ng/ml)	Urine 3 (ng/ml)	Plasma 1 (ng/ml)	Plasma 1 (ng/ml)	Plasma 1 (ng/ml)
1	89.158	79.749	88.953	27.421	18.500	39.593
2	100.220	98.473	85.606	30.355	22.576	37.499
3	101.082	102.973	78.569	28.333	20.437	35.903
4	97.806	89.257	93.535	24.619	18.987	39.893
Mean	97.066	92.613	86.666	27.682	20.125	38.222
SD	5.452	10.302	6.301	2.382	1.829	1.877
CV%	5.616	**11.124**	7.270	8.604	9.089	**4.911**

D. Spike Recovery

Human urine sample average recovery range (n=3): 80.7181-121.4601%.

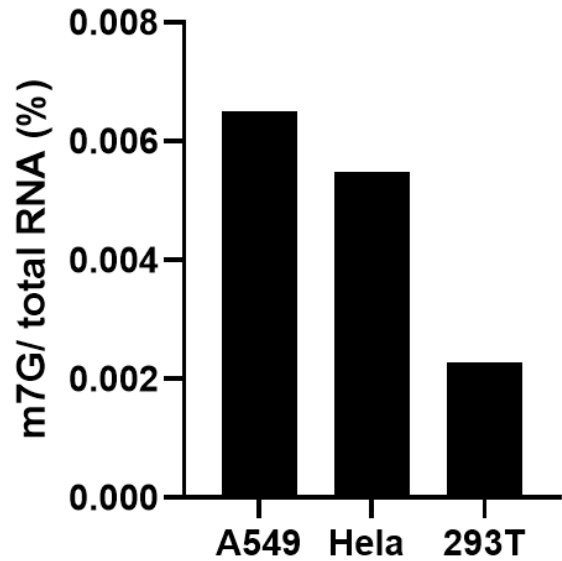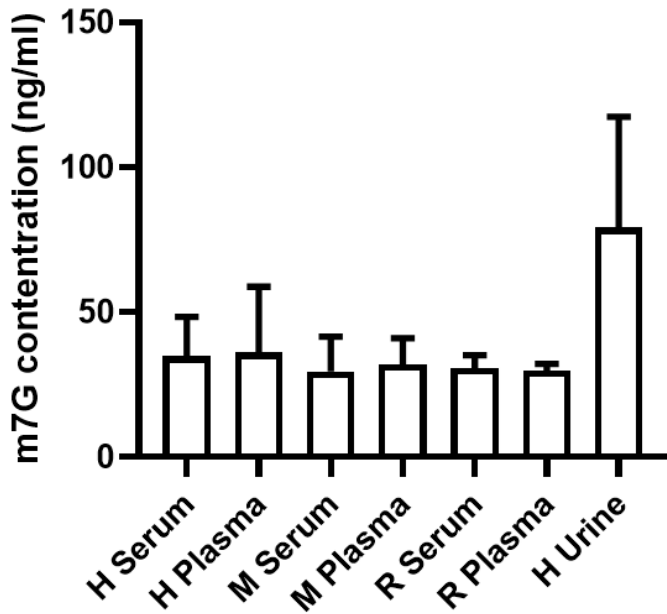| Samples	Average (ng/ml)				Recovery rate (%)		
	0	+500ng/ml	+100ng/ml	+25ng/ml	+500ng/ml	+100ng/ml	+25ng/ml
Urine 1	89.7289	661.9233	172.8547	89.7289	**99.5422**	**108.1847**	**100.2355**
Urine 2	127.9781	689.7468	192.5053	127.9781	**98.8465**	**95.8374**	**125.2409**
Urine 3	72.7187	526.6620	150.4108	72.7187	**80.7181**	**108.0571**	**121.4601**
Urine 4	98.3287	637.9921	177.0231	98.3287	**94.4455**	**105.7042**	**108.0393**
Urine 5	69.9473	626.4695	151.9570	69.9473	**96.8016**	**106.2970**	**97.1491**

E. Stability

The whole kit including coated plates, 1st antibody, 2nd antibody, standards were saved at -20℃ and 4℃ for two weeks. The standards and samples were measured.
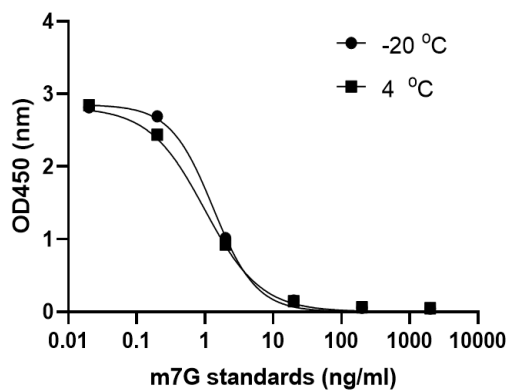
m7G STDs	-20℃-1	-20℃-2	4℃-1	4℃-2	Mean	SD	CV%
2000 ng/m	0.046	0.046	0.049	0.048	0.047	0.002	3.175
200 ng/m	0.054	0.053	0.062	0.06	0.057	0.004	7.730
20 ng/m	0.156	0.155	0.147	0.146	0.151	0.005	3.462
2 ng/m	1.012	1.021	0.927	0.923	0.971	0.053	5.458
0.2 ng/m	2.687	2.696	2.445	2.434	2.566	0.146	5.676
0.02 ng/m	2.815	2.812	2.856	2.836	2.830	0.021	0.724
0	2.699	2.714	2.562	2.554	2.632	0.086	3.268

F. Example of Test Results.

The concentration of m7G in serum and plasma samples from human (H), mouse (M) and rat (R), as well as in human (H) urine samples (n=3-6/group). The percentage of m7G in total RNA from A549, Hela and 293T cells were measured (n=3/group).

XIV. Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	o Inaccurate pipetting o Improper standard dilution	o Check pipettes. o Briefly centrifuge and dissolve the powder thoroughly by gently mixing
Low signal	o Improper preparation of standard and/or biotinylated antibody o Too brief incubation times Inadequate reagent volumes or improper dilution	o Briefly spin down vials before opening. Dissolve the powder thoroughly. o Ensure sufficient incubation time; assay procedure step 2 may be done overnight. o Check pipettes and ensure correct preparation.
Large CV	o Inaccurate pipetting Air bubbles in wells	o Check pipettes. o Remove bubbles in wells
High background	o Plate is insufficiently washed. o Contaminated wash buffer	o Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. o Make fresh wash buffer
Low sensitivity	o Improper storage of the ELISA kit o Stop solution	o Follow storage recommendations in sections IV and V. Keep substrate solution protected from light. o Add stop solution to each well before reading plate

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.