1-Methyladenosine (m^1A) ELISA Kit

—— GK- 4045

Product Manual

(Colorimetric)

*PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

FOR RESEARCH USE ONLY.
Not for use in diagnostic procedures

Introduction

Modifications to ribonucleic acids (RNAs) are integrally involved in the function, integrity, metabolism, and biosynthesis of RNA molecules. There are over 100 RNA modifications that have been discovered with the majority occurri -ng in transfer RNA (tRNA), but they can also be found in ribosomal RNA (rRNA), small nuclear RNA (snRNA), messenger (mRNA), and long non-coding RNA (ncRNA). Urinary excretion of modified nucleosides has been linked to a variety of cancers, AIDS, and other malignant disease states.

1-methyladenosine (m^1A) is a prominent reversible post-transcriptional RNA modification that influences the function and structure of tRNA and rRNA. 1-Methyladenosine occurs at positions 9, 14, and 58 of tRNA, with position 58 (m^1A58) being important to tRNA stabilization. Methyltransferases catalyze the addition of a methyl group to the first position nitrogen on the adenosine base stru cture, resulting in the addition of a positive charge to the molecule. The reaction is reversible with demethylases such as ALKBH3. 1-methyladenosine can affect ri bosomal biogenesis, antibiotic resistance in bacteria, react to environmental stress in tRNA, and interfere with Watson-Crick base pairing, which ultimately affects reverse transcription and protein translation.

Urine samples of cancer, rheumatoid arthritis, and AIDS patients have all demonstrated high levels of m^1A, which supports its role as a functional detection biomarker. Elevated serum levels of 1-methyladenosine have been detected under stress conditions. Recent 1-methyladenosine research is uncovering new roles and mechanisms involving 1-methyladenosine.

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While m^1dA in DNA is considered a form of damage leading to genomic mutations, and thus requiring repair, endogenous enzymes proliferate the m^1A modification in RNA. The m1A at position 9 (m^1A9) is known to stabilize the canonical cloverleaf motif in tRNA. In addition, the m^1A58 of tRNA is present in all eukaryotic types which may imply it is vital to molecular structure and stability . While 1-methyladenosine has been identified as an early malignant disease marker, its complete role as a modified nucleoside has yet to be elucidated.

The 1-Methyladenosine (m^1A) ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of 1-methyladenosine in urine, serum, or plasma samples. The quantity of 1-methyladenosine in unknown samples is determined by comparing its absorbance with that of a known 1-methyladenosine standard curve. The kit has a 1-methyladenosine detection sensitivity of approximately 2 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

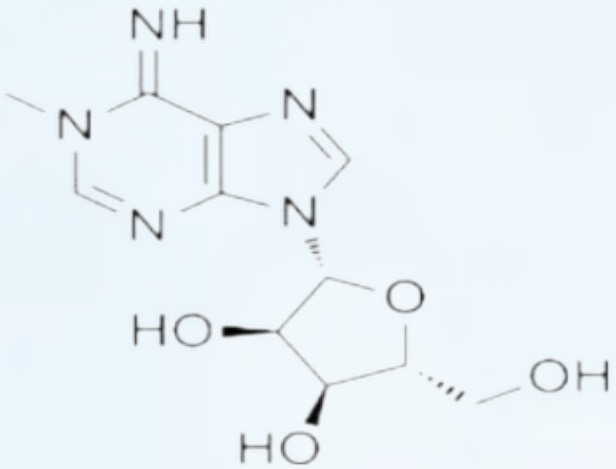

Figure 1: 1-Methyladenosine(m^1A)

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Assay Principle:

The 1-Methyladenosine (m^1A) ELISA kit is a competitive ELISA for the quantitative measurement 1- methyladenosine (m^1A). The unknown 1-methyladenosine samples or 1-methyladenosine standards are first added to a 1-methyladenosine-BSA conjugate preabsorbed microplate. After a brief incubation, an anti-1-methyladenosine monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The 1-methyladenosine content in unknown samples is determined by comparison with predetermined 1-methyladenosine standard curve.

References :

1. Borek, E., et al. (1977) Cancer Res. 37: 3362-3366.
2. Chen, W., et al. (2016) Sci Rep. 6: 31080.
3. Davis, G.E., et al. (1977) Clin. Chem. 23: 1427-1435.
4. Dominissini, D., et al. (2016) Nature 530: 441-446.
5. Macon, J.B., et al. (1968) Biochemistry 7(10): 3453-3458.6. Seidel, A., et al. (2006) Br. J. Cancer 94: 1726-1733.

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Kit Components:

Box 1 (shipped at room temperature):

1. 96-well Protein Binding Plate (Part No. 231001):
One 96-well strip plate.
2. Anti-1-Methyladenosine Antibody (Part No. 50991C):
One 10 µL vial of anti-1-Methyladenosine.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003):
One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. 1-Methyladenosine Standard (Part No. 50992C):
One 10 µL vial of 5 mg/mL 1-Methyladenosine in DMSO.

Box 2 (shipped on blue ice packs)

1. 1-Methyladenosine Conjugate (1000X) (Part No. 50993D): One 10 µL vial of 1-Methyladenosine-BSA conjugate in PBS.

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Storage:

Upon receipt, aliquot and store the Anti-1-Methyladenosine Antibody and 1-Methyladenosine Standard at -20ºC and the 1-Methyladenosine Conjugate (1000X) at -80ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents:

- 1-Methyladenosine Conjugate Coated Plate: Dilute the proper amount of 1-Methyladenosine Conjugate 1:1000 in 1X PBS depending on the number of required assays. (Example: Add 5 µL of 1-Methyladenosine Conjugate stock tube to 4.995 mL 1X PBS to coat 48 wells). Add 100 µL of this 1-methyladenosine conjugate coating solution to each well and incubate overnight at 4ºC. Remove the 1-methyladenosine conjugate coating solution and wash once with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1-2 hr at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent immediately before use.

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Note: The 1-methyladenosine-conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

● 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.

● Anti-1-Methyladenosine Antibody and Secondary Antibody, HRP Conjugate (1000X): Immediately before use dilute the Anti-1-Methyladenosine Antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve:

Prepare fresh standards by diluting the 1-Methyladenosine Standard stock from 5 mg/mL to 5 µg/mL in Assay Diluent for a 1:1000 final dilution. (Example: Add 2 µL of 1-Methyladenosine Standard stock tube to 1.998 mL of Assay Diluent). Continue preparing a dilution series of 1-methyladenosine standards in the concentration range of 0 ng/mL to 5000 ng/mL by diluting the 1-methyladenosine standards in Assay Diluent (Table 1).

Tabel 1

Standard Tubes	1-Methyladenosine Standard (μL)	1-Methyladenosine (ng/mL)	Assay Diluent (μL)
1	2	5000	1998
2	250 of Tube #1	2500	250
3	250 of Tube #2	1250	250
4	250 of Tube #3	625	250
5	250 of Tube #4	313	250
6	250 of Tube #5	156	250
7	250 of Tube #6	78	250
8	250 of Tube #7	39	250
9	250 of Tube #8	20	250
10	250 of Tube #9	10	250
11	250 of Tube #10	5	250
12	0	0	250

* Table 1. Preparation of 1-Methyladenosine Standards

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Preparation of Samples：

I. Urine

Samples containing precipitates should be centrifuged at 3000 g for 10 minutes, or filtered through 0.45 μm filter, prior to use in the assay. Perform dilutions in Assay Diluent or PBS containing 0.1% BSA as necessary.

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 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent or PBS containing 0.1% BSA as necessary.

Note: *This assay is not compatible with mouse serum or plasma due to high levels of mouse IgG that will cross react with the secondary antibody.*

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III. Plasma

Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent or PBS containing 0.1% BSA as necessary.

Note : *This assay is not compatible with mouse serum or plasma due to high levels of mouse IgG that will cross react with the secondary antibody.*

IV. Cell or Tissue RNA Samples

1. Purify RNA from cell or tissue samples by a desired method or commercial RNA Extraction kit.
2. Dissolve purified RNA in nuclease free water at 1-5 mg/mL.
3. Remove any RNA secondary structure by incubating the sample at 95ºC for 5 minutes and rapidly chilling on ice.
4. Digest RNA sample to nucleosides by incubating the denatured RNA with 5-20 units of nuclease P1 (previously reconstituted in the manufacturer's recommended buffer) for 2 hrs at 37ºC in a final concentration of 20 mM Sodium Acetate, pH 5.2.

5. Add 5-10 units of alkaline phosphatase (previously reconstituted in the manufacturer's recommended buffer) plus sufficient Tris buffer to a final concentration of 100 mM Tris, pH 7.5, and incubate for 1 hr at 37ºC.
6. Centrifuge the reaction mixture for 5 minutes at 6000 x g and collect the supernatant for use in the ELISA.

Assay Protocol :

1. Prepare and mix all reagents thoroughly before use. Each 1-methyladenosine sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or 1-Methyladenosine Standard to the wells of the 1- Methyladenosine Conjugate Coated Plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted Anti-1-Methyladenosine Antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

5 . Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to all wells.

6. Incubate at room temperature for 1 hour on an orbital shaker.

7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.

8. Warm Substrate Solution to room temperature. Add 100 L of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

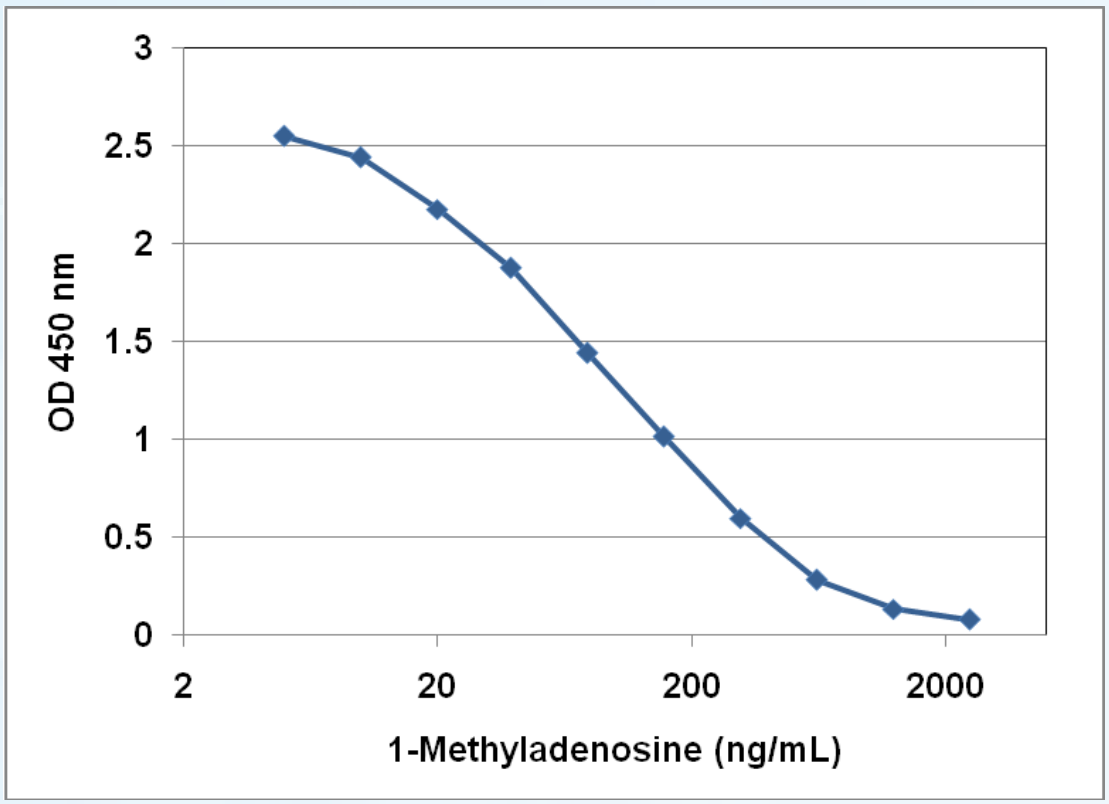
Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results：

The following figures demonstrate typical 1-Methyladenosine ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.



* Figure 2: 1-Methyladenosine ELISA Standard Curve.

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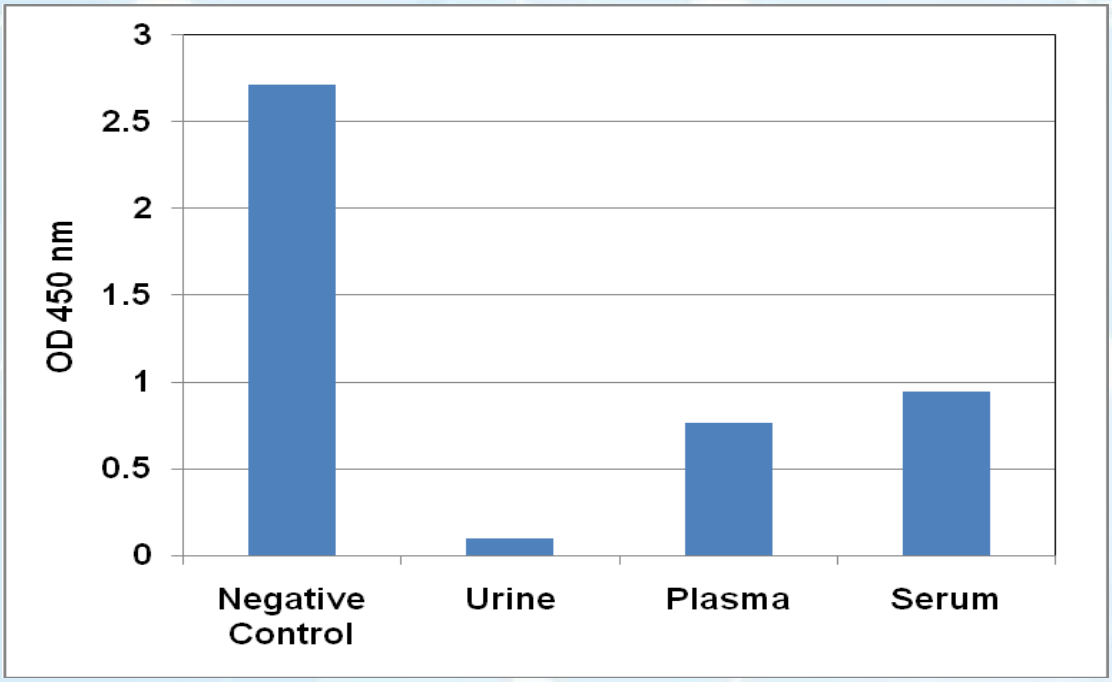

Figure 3: 1-Methyladenosine levels in human urine, plasma, and serum. Undiluted human samples were tested according to the Assay Protocol instructions.

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