1-methyladenosine ELISA Kit

1-methyladenosine ELISA Kit is an Enzyme Immunoassay kit for the quantification of 1-methyladenosine in urine, serum, cell or tissue lysate.

Catalog number: ARG83376

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

1-methyladenosine (m1A) 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 (m1A58) being important to tRNA stabilization. Methyltransferases catalyze the addition of a methyl group to the first position nitrogen on the adenosine base structure, resulting in the addition of a positive charge to the molecule. The reaction is reversible with demethylases such as ALKBH3. 1-methyladenosine can affect ribosomal 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 m1A, 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. While m1 dA in DNA is considered a form of damage leading to genomic mutations, and thus requiring repair, endogenous enzymes proliferate the m1A modification in RNA. The m1A at position 9 (m1A9) is known to stabilize the canonical cloverleaf motif in tRNA. In addition, the m1A58 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.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. After coated 1-Methyladenosine Conjugate onto a microtiter plate, 1-Methyladenosine of a sample competes with a 1-Methyladenosine- antibody for binding to the coated Conjugate. After incubation the unbound antibody is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of 1-Methyladenosine in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of 1-Methyladenosine. 1-Methyladenosine concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard at ≤ -20°C, 1000X 1-methyladenosine Conjugate at -80°C. Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
1000X 1-methyladenosine Conjugate	1 vial (10 µL)	-80°C
Standard	1 vial (10 µL; 5 mg/mL)	-20°C
1000X 1-methyladenosine Antibody	1 vial (10 µL)	4°C
1000X HRP-Streptavidin Solution	1 vial (20 µL)	4°C
Diluent Buffer	50 mL	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)
- RNA extraction kit and 1X PBS

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Standard at ≤ -20°C, 1000X 1-methyladenosine Conjugate at-80°C. Store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months.

Cells or Tissues Lysate: RNA must first be isolated before assaying.

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 95°C 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 37°C 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 37°C.
6. Centrifuge the reaction mixture for 5 minutes at 6000 x g and collect the supernatant for use in the ELISA

REAGENT PREPARATION

- 1X 1-methyladenosine Conjugate: 10 minutes before use, dilute 1000X 1-methyladenosine Conjugate into 1X PBS to yield 1X 1-methyladenosine Conjugate.
- 1-Methyladenosine Conjugate Coated Plate: Add 100 µL 1-methyladenosine conjugate coating solution to each well and incubate overnight at 4°C.

  After incubation, 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 4°C and remove the Assay Diluent immediately before use.
- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer.

- **1X 1-methyladenosine Antibody:** 10 minutes before use, dilute **1000X** 1-methyladenosine Antibody into Diluent Buffer to yield **1X** 1-methyladenosine Antibody.
- **1X HRP-Streptavidin Solution:** 10 minutes before use, dilute **1000X** HRP-Streptavidin Solution into Diluent Buffer to yield **1X** HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Standard:** Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	1-methyladenosine (μg/mL)	Diluent Buffer (μL)	Standard (μL)
S1	5000	1998	2 (5 mg/mL Stock)
S2	2500	250	250 of S1
S3	1250	250	250 of S2
S4	625	250	250 of S3
S5	313	250	250 of S4
S6	156	250	250 of S5
S7	78	250	250 of S6
S8	39	250	250 of S7
S9	20	250	250 of S8
S10	10	250	250 of S9
S11	5	250	250 of S10
S0	0	250	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

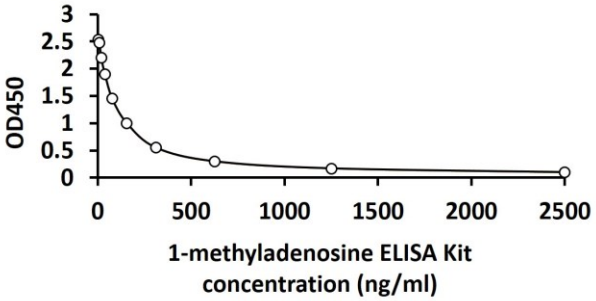
1. Add **50 µL** of **Samples, Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **10 min** at **RT**.
3. Add **50 µL** of **1X 1-methyladenosine antibody** into each wells.
4. Cover the plate and incubate for **1 hour** at **RT**.
5. Aspirate each well and wash, repeating the process 2 time for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
7. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
8. Aspirate each well and **wash** plate **as step 5**.
9. Add **100 µL** of **TMB Substrate** in each well.
10. Incubate for **5-30 mins** at **room temperature** in the dark.
11. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
12. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the 1-methyladenosine ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

2 ng/ml

Assay Range

5- 5000 ng/ml