Version 21-04/25

User's Manual

mRNA (nucleoside-2'-O-)-methyltransferase ELISA Kit

REF **DEIA-BZ001**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This assay kit is capable for the quantitative determination mRNA (nucleoside-2'-O-)-methyltransferase.

General Description

mRNA (nucleoside-2'-O-)-methyltransferase is a methyltransferase encoded by the DNA of the vaccinia virus. This enzyme uses S-Adenosylmethionine (SAM) as a methyl donor to add methyl groups to the 2'-O site of the first nucleotide at the 5' end of RNA adjacent to the cap structure to form mRNA with Cap1 structure. Cap1 structure can enhance the efficiency of mRNA translation and reduce the immunogenicity of mRNA structure itself, thus helping to improve the expression level of encoded protein after mRNA transfection. mRNA (nucleoside-2'-O-)-methyltransferase can specifically recognize the m7GpppN (cap0) structure but does not act on RNA with the 5' end pN, ppN, pppN or GpppN.

Principles of Testing

The kit is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay. Samples and standards are captured by a solid bound specific antibody. HRP conjugated tracer antibody will bind to captured mRNA (nucleoside-2'-O-)-methyltransferase. HRP conjugate will react with the substrate, tetramethylbenzidine (TMB). The enzyme reaction is stopped by the addition of stop solution. The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (log) versus the corresponding concentrations of the mRNA (nucleoside-2'-O-)-methyltransferase standards (log). The concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

Reagents And Materials Provided

BOX 1:

1. 2'-O-Methyltransferase standard (8,000 ng/mL), 100 µL

BOX 2:

1. 12 Microtiter strips, pre-coated, 1 plate
2. Sample Diluent, 12 mL
3. ELISA detection antibody diluent, 12 mL
4. Enzyme-labeled detection antibody (100×), 120 µL
5. Washing Solution (20×), 30 mL
6. Substrate Solution, 12 mL
7. Stop Solution, 6 mL
8. Parafilm, 3 sheets

Materials Required But Not Supplied

1. Deionized water
2. Microplate Mixer
3. Plate washer
4. Incubator
5. Timer
6. Calibrated micropipettes and disposable tips
7. Calibrated ELISA plate reader capable of measuring absorbance at 450 nm

Storage

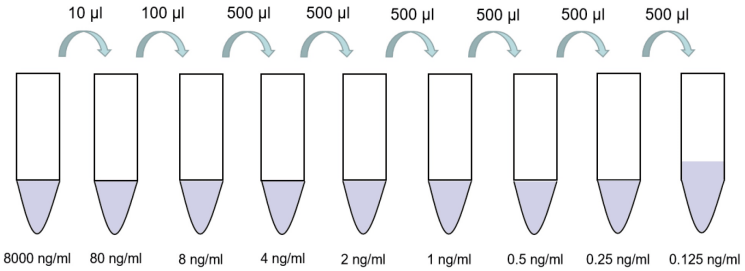
BOX 1, store at -30—-15°C; BOX 2, store at 2—8°C.

Do not freeze kits and store away from light. The expiry date is specified on outer labels.

Reagent Preparation

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

1. Prepare wash buffer by mixing 30 mL of 20× concentrated wash buffer with 570 mL of distilled or de-ionized water. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20× wash buffer with 19 parts of distilled or de-ionized water.
2. The standard is reconstituted by pipetting the amount of sample dilution buffer. Use the standard vial as Tube 1 in figure. Prepare each standard in polypropylene tubes by 2-fold serial dilution of the reconstituted standard with Sample dilution buffer as shown in figure. After reconstitution the standard cannot be stored for repeated use.



3. Dilute the enzyme-labeled detection antibody (100×) to 1× with the enzyme-labeled detection antibody diluent, invert and mix at least 30 times. For example, 100 µL of 100× enzyme-labeled detection antibody is diluted with 9.9 mL of enzyme-labeled detection antibody diluent.

Assay Procedure

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame.

Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.

2. Transfer 100 µL in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
3. Cover the tray and tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 1 hour at 37°C.
5. Wash the plates 4 times with wash buffer using a plate washer or as follows*:

   a. Carefully remove the plate sealer, avoid splashing.

   b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.

   c. Add 300 µL of wash buffer to each well, wait 30 seconds, empty the plate as described in 5b.

   d. Repeat the washing procedure 5b/5c three times.

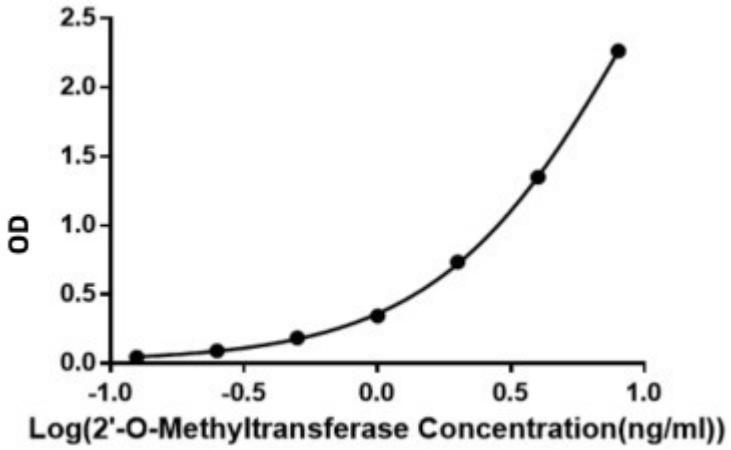
   e. Empty the plate and gently tap on thick layer of tissues.
6. Add 100 µL of diluted 1× enzyme-labeled detection antibody to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
7. Cover the tray and incubate the tray for 1 hour at 37°C.
8. Repeat the wash procedure described in step 5.
9. Add 100 µL of substrate solution to each well, using the same pipetting order as applied in step2. Do not touch the side or bottom of the wells.
10. Cover the tray and incubate the tray for 10 minutes away from light at 37°C.
11. Stop the reaction by adding 50 µL of stop solution with the same sequence and timing as used in step 9. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
12. Read the plate within 10 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

*) In case plate washer is used, please note: use of a plate washer can result in higher background and decrease in sensitivity. We advise validation of the plate washer with the manual procedure. Make sure the plate washer is used as specified for the manual method.

Calculation

1. Calculate the mean absorbance for each set of duplicate standards, control and samples.
2. If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
3. Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis (logarithmic scale) versus the corresponding concentration on the horizontal (X) axis (logarithmic scale).
4. Lowest limit of quantification (LOQ) = 125 pg/mL, below 125 pg/mL reported as <125 pg/mL. If the OD value of the sample is higher than the upper limit of the standard curve, it should be re-measured after appropriate dilution. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve



Precision

	Intra-Assay			Inter-Assay		
sample	1	2	3	1	2	3
n	10	10	10	30	30	30
average	3.79	0.84	0.21	3.93	0.91	0.23
SD	0.16	0.04	0.01	0.29	0.06	0.02
CV	4%	5%	5%	7%	6%	7%

Sensitivity

LOD: 8.15 pg/mL, LOQ: 125 pg/mL

Specificity

Specific

Recovery

Sample (n=10)	Average Concentration (ng/mL)	Average Recovery (%)	Recovery Range (%)
4 ng/mL	4.09	102	93 - 115
1 ng/mL	0.89	89	83 - 98
0.25 ng/mL	0.23	91	83 - 98

Precautions

1. Please strictly follow the instructions for the storage and usage of each component.
2. Please strictly check the validity period and packaging of the kit before use. Do not use for experimental operations if expiry time expires or packaging is broken.
3. This product is for scientific research only.
4. Disposable gloves and protective items specified by the laboratory must be worn during operation. The waste liquid and equipment after testing must be treated harmlessly in accordance with the regulations of the local government and relevant countries.