

Murine RNase Inhibitor ELISA Kit

Product description

RNase inhibitor is a specific inhibitor of ribonuclease (RNase) found in the human placenta, which specifically inactivates RNase by binding to it non-covalently to form a complex that inactivates RNase.

This kit uses the principle of double-antibody sandwich enzyme-linked immunosorbent assay (sandwich ELISA) to detect the residual Murine RNase Inhibitor in samples. First, add the Murine RNase Inhibitor standard and test sample to the Anti-Murine RNase Inhibitor coated microtiter strips (36707-A), then add the diluted biotin labeled Anti-Murine RNase Inhibitor antibody (36707-C), and finally add Streptavidin-HRP (36707-D) to form an antibody + antigen + antibody-Biotin + SA-HRP complex. Subsequently, add TMB substrate (36707-H) into the complex to observe color reaction after washing the complex. TMB is converted into blue under the catalysis of HRP enzyme and finally converted into yellow in the presence of acid, and the shade of color is positively correlated with the amount of Murine RNase Inhibitor in the sample.

The detection range of this kit is 2~128 ng/mL; the lower detection limit is 1.5 ng/mL.

Specifications

Cat.No.	36707ES48 / 36707ES96
Size	48 T / 96 T

Components

Components No.	Name	36707ES48	36707ES96
36707-A	Anti-Murine RNase Inhibitor coated microtiter strips	48 T	96 T
36707-B*	Standard: Murine RNase Inhibitor	1 vial	2 vial
36707-C	Detection Antibody: Biotin-conjugated Antibodies	60 μ L	120 μ L
36707-D	Streptavidin-HRP	20 μ L	40 μ L
36707-E	Dilution Buffer 1	25 mL	45 mL
36707-F	Wash Buffer Concentrate (20 $\times$)	25 mL	50 mL
36707-G	Dilution Buffer 2	15 mL	30 mL
36707-H	TMB Substrate	8 mL	15 mL
36707-I	Stop Solution	5 mL	10 mL
36707-J	Plate Sealer	3 each	5 each

*The Standard (36707-B) is Lyophilized powder.

Storage

This product* should be stored at 2°C ~8°C. Unopened product is valid for one year.

*Upon receipt of the kit, please check whether all components are complete and immediately store them in corresponding condition.

Instructions

1. Preparations before the experiment

1) Materials required but not provided:

- a. Graduated cylinder, Beaker, Centrifuge tube of different volumes;
- b. Dust-free paper (used for plate washing)
- c. Sterile pipette tips;
- d. Pre-treated plates;
- e. Deionized water or double distilled water.

2) Instruments and equipment required but not provided:

- a. 37°C incubator;
- b. Plate washer;
- c. Timer, Vortex and Centrifuge;
- d. Single channel or multi-channel pipettes;
- e. 2°C ~8°C refrigerator;
- f. Microplate reader (such as Molecular Devices: M and i series) which with can measure the absorbance at 450 nm (also with the reference wavelength at 630nm).

2. Assay method

1) Reagent preparation

All reagent components and samples to be tested need be balanced to the room temperature before use and be prepared freshly for use.

a. Preparation of 1×Wash Buffer:

Equilibrate the Wash Buffer Concentrate (20 ×) to room temperature and dissolve it fully without crystals. Then dilute it by 1:20 with the the Deionized water or double distilled water according the requirement. For example, mix well and transfer 50 mL of Wash Buffer Concentrate (20 ×) into container, and then bring the volume to 1 L with Deionized water or double distilled water. If crystallization occurs in Wash Buffer Concentrate (20 ×), warm it in a water bath at 50°C until the crystallization completely disappears.

b. Preparation of Detection Antibody:

Centrifuge at 10,000 rpm for 20 sec before use, and then dilute the detection antibody by 1:100 with **Dilution Buffer 2** to make a working solution.

c. Preparation of Streptavidin-HRP:

Centrifuge at 10,000 rpm for 20 sec before use, and then dilute the Streptavidin-HRP by 1:500 with **Dilution buffer 2** to make a working solution.

2) Standard curve preparation:

Prepare 8 clean 1.5 mL centrifuge tubes and label them sequentially according to the standard concentration. Take 1 vial lyophilized standard and dissolve the standard powder with Dilution buffer 1 marked in volume on the tube. Let the tube stand for about 10 min and gently mix well, the final concentration is 512 ng/mL.

450 μL of Dilution Buffer 1 was added to the first centrifuge tube and 300 μL of Dilution Buffer 1 was added to each of the remaining centrifuge tubes. Take 150 μL of the dissolved and mixed 512 ng/mL standard, add it to the first centrifuge tube, mix it well and label it as 128 ng/mL, then take 300 μL to the next centrifuge tube labeled with the concentration, mix it well and carry out a series of 2-fold gradient dilutions, with the highest concentration labeled

as 128 ng/mL at the beginning and the lowest as 2 ng/mL, which can be formulated according to the following preparation method to proceed. The standard curve should be prepared for each test, and the standard curves of different kits and different times should not be mixed. When testing samples, the amount of standard required for each well is 100 μ L, note that the preparation volume should be higher than the required volume to avoid under-use.

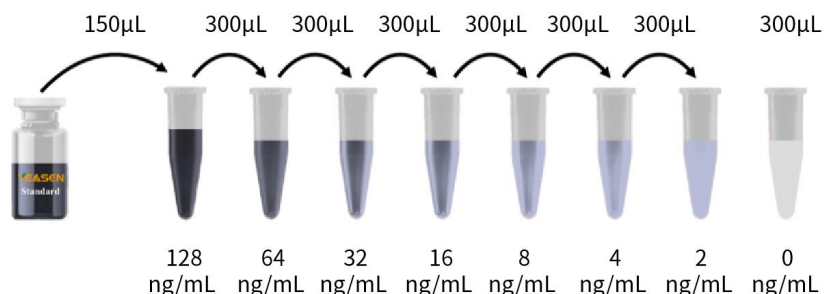


Figure 1 Simplified flow chart of standard configuration

Vial	Volume of Dilution Buffer 1 (μ L)	Volume of Standard (μ L)	Final concentration of Standard (ng/mL)
A	450	150 (512 ng/mL)	128
B	300	300 A	64
C	300	300 B	32
D	300	300 C	16
E	300	300 D	8
F	300	300 E	4
G	300	300 F	2
H	300	0	0

Table 1 Murine RNase Inhibitor standard preparation

3) Test samples preparation:

Dilute the test samples with certain dilution factor by using **Dilution Buffer 1**. The specific dilution of the sample needs to be determined by spiking the sample and evaluating the spiking recovery and dilution linearity to determine the appropriate dilution.

4) Experiment flow

All reagent components and samples to be tested need be balanced to the room temperature before use. It is strongly recommended that all standards and test samples be assayed in duplicate.

a. Reagents and samples preparation:

Prepare required reagents, diluted standards and samples.

b. Strip Determination:

Calculate the strips required for test samples and standards, take strips from the foil bag, put the remaining strips back into the foil bag, seal the bag, and store at 2°C ~8°C.

c. ELISA plate washing:

Wash the plate for 3 times with 1×Wash Buffer (300 μ L/well), and pat the ELISA plate dry. The plate washing has an important impact on the test results, make sure that no wash solution remains in the plate.

d. Samples incubation:

Add standards and test samples, 100 μ L/well, ensure that the sample adding is completed within 15 min, and

incubate at 37°C for 1 h.

e. ELISA plate washing:

Discard the liquid in the wells, wash the plate with 1×Wash Buffer (300 μ L/well) for 5 times, and pat the ELISA plate dry.

f. Detection antibody incubation:

Add the pre-diluted detection antibody into the ELISA plate, 100 μ L/well, and incubate at 37°C for 1 h.

g. ELISA plate washing:

Discard the liquid in the wells, wash the plate with 1×Wash Buffer (300 μ L/well) for 5 times, and pat the ELISA plate dry.

h. HRP-conjugated Streptavidin incubation:

Add the pre-diluted HRP-conjugated Streptavidin into the ELISA plate, 100 μ L/well, and incubate at 37°C for 40 min.

i. ELISA plate washing:

Discard the liquid in the well, wash the plate with 1×Wash Buffer (300 μ L/well) for 5 times, and pat the ELISA plate dry.

j. Color reaction:

Balance the TMB solution to room temperature 10 min before use, add the TMB to the ELISA plate, 100 μ L/well, and incubate at 37°C for 15 min away from light.

k. Stopping:

Add stop solution to the plate, 50 μ L/well, and shake the plate gently until the color is uniform.

l. Reading:

Read the absorbance at 450 nm/630 nm within 20 min. 450 nm as the detection wavelength, and 630 nm as the reference wavelength.

5) Result Analysis

a. If the OD value of the sample exceeds the maximum peak of the standard curve, the sample should be diluted and re-measured.

b. Standard curve drawing:

The standard curve is drawn with the concentration of the standard as the abscissa and the absorbance value of OD_{450nm-630nm} as the ordinate. A variety of mapping and statistical software can be used to aid in drawing standard curves and calculating unknown sample concentrations. The four-parameter fitting method often has better curve fitting effect, and other methods such as linear and double-logarithm method may also obtain better fitting results, which need to be analyzed according to specific experimental data. Finally, the residual Murine RNase Inhibitor concentration in the sample will be calculated according to the standard curve and the dilution ratio of the sample.

c. The typical Standard Curve is as follows (the following standard curve is for reference only, and the sample content should be calculated based on the standard curve generated in the same experiment) :

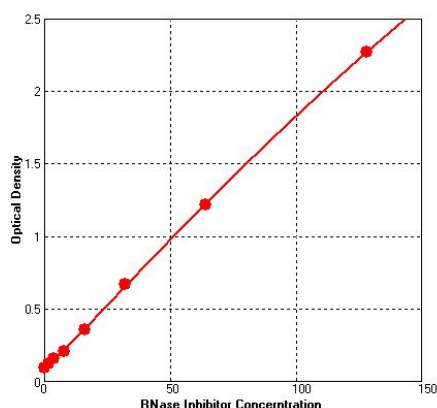


Figure 2 Typical standard curve

Standard Conc. (ng/mL)	OD		Mean OD	Calibration value
128	2.322	2.222	2.272	2.271
64	1.223	1.216	1.220	1.227
32	0.689	0.656	0.673	0.657
16	0.359	0.358	0.359	0.367
8	0.206	0.218	0.212	0.226
4	0.167	0.157	0.162	0.158
2	0.124	0.128	0.126	0.126
0	0.091	0.098	0.095	/

Table 2 Typical standard curve data

6) Experiment flow chart

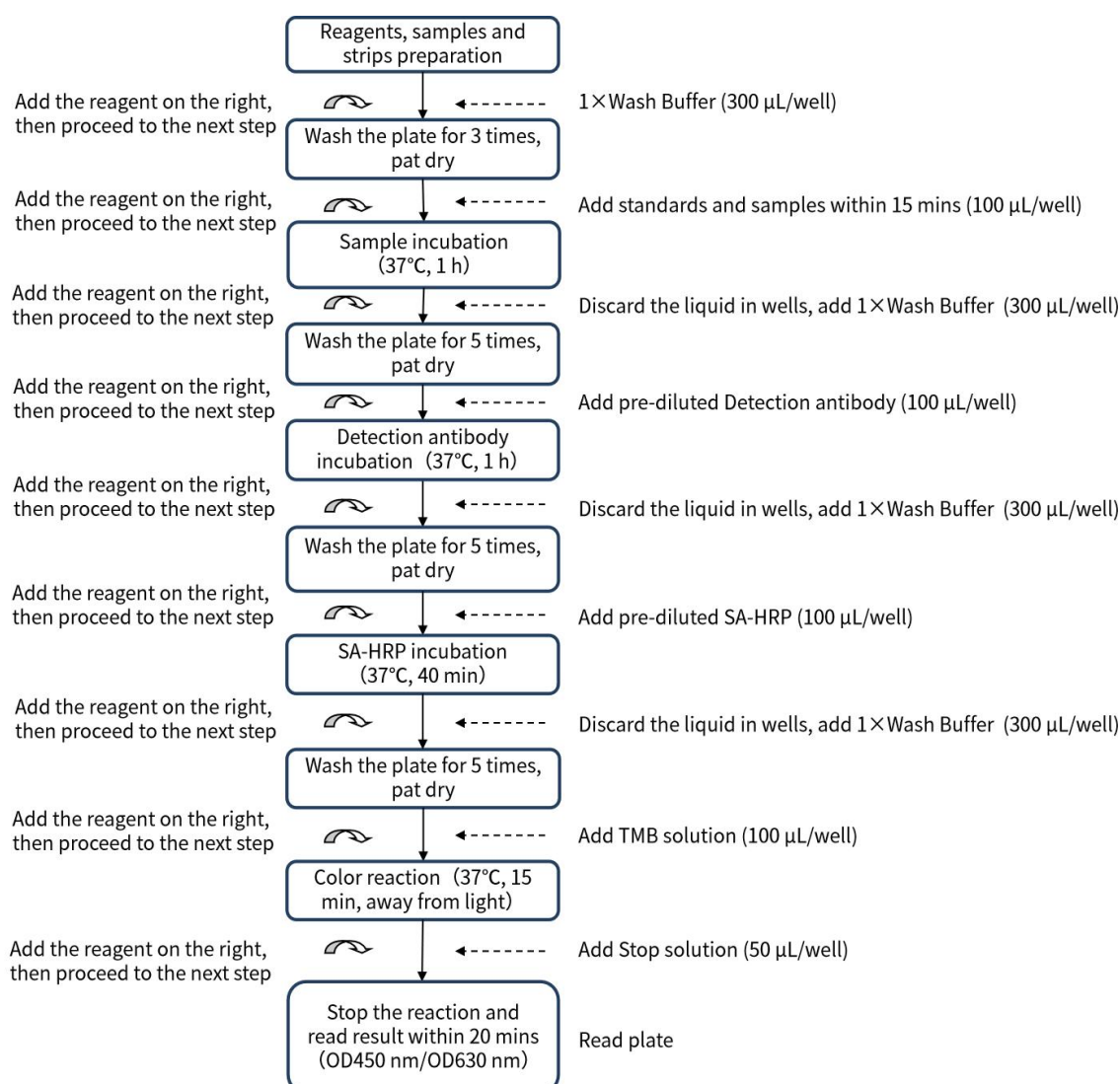


Figure 3 Experiment flow chart

Notes

1. Please read this manual carefully before using this kit. The kit needs to be used up within its shelf life.
2. Mixing of related reagents from different batches is prohibited.

3. The experiment should be conducted in a standardized manner, vortex and mix well for each reagents and samples before use.
4. The kit is designed for detecting the target antigens and samples marked in the instructions only. Other applications need to be designed and verified by the user, and the reliability and accuracy should be evaluated based on the results.
5. For your safety and health, please wear personal protective equipment (PPE), such as laboratory coats and disposable gloves, when operating with this product.
6. This product is for research use only.