Version 28-04/25

User's Manual

RNase Inhibitor ELISA Kit

REF **DEIANS033**

Σ **96T**

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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

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PRODUCT INFORMATION

Intended Use

This kit employs double-antibody sandwich ELISA to detect the RNase Inhibitor in samples.

Principles of Testing

This kit employs double-antibody sandwich ELISA to detect the content of RNase Inhibitor. Coat the microplate with anti-RNase Inhibitor antibodies, add the RNase Inhibitor standard and the sample to be tested into the solid-phase antibody microplate, then add detection antibodies (Anti-RNase Inhibitor), and finally add FC-secondary antibodies to form complexes. Add the TMB substrate solution after washing to develop color.

Reagents And Materials Provided

1. RNase Inhibitor Coated Plate, 8×12, ready to use.
2. Anti-RNase Inhibitor, 150μL
3. Streptavidin HRP, 150μL
4. RNase Inhibitor Standard, 30μL (0.235mg/mL)
5. Sample Diluent Buffer, 60mL, ready to use.
6. Antibody Diluent Buffer, 12mL, ready to use.
7. Enzyme Conjugate Diluent Buffer, 12mL, ready to use.
8. 20×PBST Wash Buffer, 50mL
9. TMB Substrate, 11mL, ready to use.
10. Stop Solution, 7mL, ready to use.

Materials Required But Not Supplied

1. Deionized or distilled water
2. Shaker
3. Micropipettes and compatible sterile tips
4. Microplate reader
5. Vortex
6. Filter paper

Storage

Anti-RNase Inhibitor, Streptavidin HRP and RNase Inhibitor Standard: store at -20°C.

Other components: store at 2-8°C. Protect the kit from light.

The shelf life is 12 months.

Reagent Preparation

Note: Take out the kit from the refrigerator and the standard from the freezer, and equilibrate them at room temperature (18-25°C) for at least 30 minutes.

1. 1×PBST Wash Buffer: Dilute the 20×PBST Wash Buffer with deionized water in the volume ratio of 1:20.

2. Anti-RNase Inhibitor Solution: Dilute the Anti-RNase Inhibitor with Antibody Diluent Buffer in the volume ratio of 1:100.

3. Streptavidin HRP Solution: Dilute the Streptavidin HRP with Enzyme Conjugate Diluent Buffer in the volume ratio of 1:100.

4. RNase Inhibitor Standard Solution and Sample: Dilute the RNase Inhibitor Standard and Sample with Sample Diluent Buffer.

No.	Concentration of Standard (ng/mL)	Volume of Standard (μL)	Volume of Sample Diluent Buffer (μL)	Total Volume (μL)	Concentration of Prepared Standard (ng/mL)	Remaining volume (μL)
Pre-1	235000	5	45	50	23500	16
Pre-2	23500	34	590	624	1280	324
7	1280	300	300	600	640	300
6	640	300	300	600	320	300
5	320	300	300	600	160	300
4	160	300	300	600	80	300
3	80	300	300	600	40	300
2	40	300	300	600	20	600
1	/	/	300	300	0	300

Assay Procedure

1. Before use, mix all reagents thoroughly to avoid air bubbles.
2. Take out a required number of pre-coated microplate strips, keep the remainder in a ziplock bag and put it back at 2-8°C.
3. Add the sample, RNase Inhibitor Standard Solution and negtive control at 100 μl per well into the pre-coated microplate. Seal the plate with a sealing film and incubate it in a 37°C, 200-300rpm thermostatic incubator for 1 hour.
4. Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 μl of 1×PBST Wash Buffer into each well, and leave it to soak for 30 seconds. Wash the plate 4

times, and remove all of the residual liquid to the extent possible in the last washing.

5. Add the Anti-RNase Inhibitor Solution at 100 µl per well into the pre-coated microplate. Seal the plate with a sealing film and incubate it in a 37°C, 200-300rpm thermostatic incubator for 1 hour.
6. Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 µl of 1×PBST Wash Buffer into each well, and leave it to soak for 30 seconds. Wash the plate 4 times, and remove all of the residual liquid to the extent possible in the last washing.
7. Add the Streptavidin HRP Solution at 100 µl per well into the pre-coated microplate. Seal the plate with a sealing film and incubate it in a 37°C, 200-300rpm thermostatic incubator for 1 hour.
8. Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 µl of 1×PBST Wash Buffer into each well, and leave it to soak for 30 seconds. Wash the plate 4 times, and remove all of the residual liquid to the extent possible in the last washing.
9. Add the TMB Substrate into the plate at 100 µl per well, seal the plate with a sealing film, and incubate it in a 25°C thermostatic incubator for 15 minutes (keep away from light).
10. Add the stop solution at 50 µl per well, gently mix well and then use a microplate reader to determine the absorbance per well at a single wavelength of 450 nm within 10 minutes. (Select the microplate reader main wavelength 450nm, reference wavelength 630nm)

Calculation

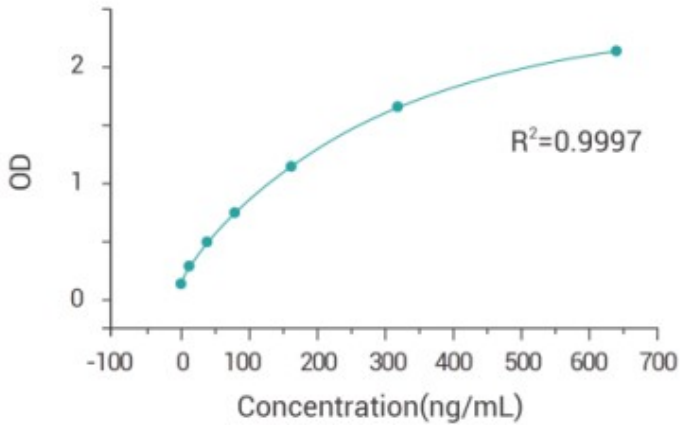
Plot the calibration curve by four-parameter fitting with the logarithm of the concentration of the standard as the x-coordinate and the OD value as the y-coordinate. If replicate wells are set, the calculation should be based on the mean value.

Typical Standard Curve

OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail)

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
640	2.213	2.110	2.162
320	1.661	1.641	1.651
160	1.181	1.143	1.162
80	0.743	0.755	0.749
40	0.486	0.478	0.482
20	0.298	0.292	0.295
0	0.151	0.15	0.151

A standard curve will be obtained by a four-parameter fit of the theoretical concentration of the standard to the corresponding OD value (as shown in the figure below).



Precision

CV%<10%

Detection Range

20-640ng/mL

Detection Limit

This reagent is only used to detect the content of RNase Inhibitor in samples.

Sensitivity

5ng/mL

Precautions

1. If the sample to be tested is a purified product, it is usually recommended to test the original solution or dilute it by 2 times. For the first assay, at least 3 serial dilutions are recommended to produce at least one diluted sample within the range of the standard curve. Diluents should be mixed well before further analysis or dilution. Each sample was analyzed in duplicate to determine the correct RNase Inhibitor residue value in the original sample.
2. Reagents should be stored according to label instructions and equilibrated at room temperature before use.
3. Before the plates are used, please equilibrate to room temperature before opening the outer packaging bag. Slats not used in the experiment should be immediately returned to the packaging and sealed tightly. It can be stored at 4°C for one month. The remaining unused reagents should be packed or covered.
4. The volume of the standard, biotin and enzyme conjugates is very small. Please centrifuge briefly before use to allow the liquid on the tube wall or cap to settle to the bottom of the tube.
5. Please use disposable tips during the experimental operation to avoid cross-contamination.
6. Check all reagents in the kit before use. Reagent dilution, sample addition and termination of reaction

should be thoroughly mixed or shaken, which is particularly important for experimental results.

7. The washing solution remaining in the reaction well during washing should be fully patted dry on a clean paper towel until the watermark is no longer visible. Do not put a paper towel directly into the reaction well to absorb water.
8. The substrate chromogenic solution is sensitive to light, avoid prolonged exposure to light, and avoid contact with metals to affect the results.
9. This product is a one-time use kit, please use it within the validity period.