Creative Diagnostics®

Version 19-11/24

User's Manual

RNase Inhibitor ELISA Detection Kit

REF **DEIA-PX0010**

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

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PRODUCT INFORMATION

Intended Use

This kit is applicable to rapid detection of RNase Inhibitor content in samples.

Principles of Testing

This product uses a double-antibody sandwich method to detect RNase Inhibitor in samples, which involves pre-coating microplate wells with an antibody specific to RNase inhibitor, and then adding the standard and test sample into the coated reaction wells for incubation. RNase Inhibitor present will quantitatively bind to the antibody in the microplate, and the test procedure is to remove the unbound complex by washing, add anti-RNase Inhibitor monoclonal antibody (detection antibody), and finally add FC secondary antibody (enzyme conjugate) to form an antibody-antigen-antibody-secondary antibody complex, and indicate the protein content in the sample by observing the intensity of TMB color development. Please read the instructions for use carefully and check the components of the kit before use.

Reagents And Materials Provided

1. RNase Inhibitor Coated Plate, 8 wells × 12 strips, Ready-to-use
2. Anti-RNase Inhibitor (detection antibody), 150 µL × 1 vial, 1:100 dilute with Antibody Diluent Buffer
3. Streptavidin HRP (enzyme conjugate), 150 µL × 1 vial, 1:100 dilute with Enzyme Conjugate Diluent Buffer
4. RNase Inhibitor Standard, 30 µL × 1 vial, (0.359 mg/mL), Operate as per the recommended dilution procedure
5. Sample Diluent Buffer, 60 mL× 1 bottle, Ready-to-use
6. Antibody Diluent Buffer, 12 mL× 1 bottle, Ready-to-use
7. Enzyme Conjugate Diluent Buffer, 12 mL× 1 bottle, Ready-to-use
8. 20×PBST Wash Buffer (20×PBST), 50 mL× 1 bottle, 1:20 dilute with deionized water
9. TMB Substrate, 11 mL× 1 bottle, Ready-to-use
10. Stop Solution, 7 mL× 1 bottle, Ready-to-use
11. Plate Sealer, 5 pieces, Ready-to-use
12. Instructions for Use, 1 copy, Ready-to-use

Materials Required But Not Supplied

(1) Plate reader

(2) Thermostat plate shaker

(3) Micro pipette and tips

(4) Deionized water

(5) Unused filter paper

(6) Vortex shaker

Storage

Detection antibody, enzyme conjugate, and standard should be stored at -18°C, and other components should be stored at 2 ~ 8°C away from light. The shelf life is 12 months.

Reagent Preparation

1. Temperature equilibration: Transfer reagents to be used to room temperature (18 ~ 25°C) environment and equilibrate the temperature for 30 minutes.

2. Preparation:

a. 1× PBS-T Wash Buffer: Calculate the volume of working buffer required, measure an appropriate amount of 20× PBS-T Wash Buffer, dilute with deionized water at 1:20, and mix well for later use.

b. Detection antibody working solution: Calculate the volume of working solution required for the test, dilute an appropriate amount of biotin antibody with diluent in a ratio of 1:100, and mix well for later use.

c. Enzyme conjugate working buffer: Calculate the volume of working solution required for the test, dilute an appropriate amount of enzyme conjugate with enzyme conjugate diluent in a ratio of 1:100, and mix well for later use.

d. The standard and test samples should be diluted with the Diluent Buffer.

3. Dilution of standard:

Vial No.	Standard solution concentration (ng/mL)	Standard solution volume (μL)	Diluent Buffer volume (μL)	Total volume (μL)	Final concentration (ng/mL)	Remaining volume (μL)
Pre-1	359000	5	71.4	76.4	23500	37.4
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. Mix all reagents well before use to avoid bubbles.
2. Confirm the number of stripe plates required based on the number of experimental wells. Put remaining strip plates back to aluminum foil bags with desiccants and seal the bag.
3. Loading: Add standard, sample dilution working buffer, and negative control into respective wells at 100 μL/well. Seal the microplate with microplate sealer and incubate in a 37°C constant temperature shaking incubator at 200-300 rpm for 60 minutes.
4. Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 μL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on the filter paper after each washing.
5. Addition of detection antibody working buffer: Add 100 μL of detection antibody working solution into each well, seal the microplate with microplate sealer, and incubate in a 37°C constant temperature shaking

incubator at 200-300 rpm for 60 minutes.

6. Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.
7. Addition of enzyme conjugate working buffer: Add 100 µL of enzyme conjugate working buffer to each well. After sealing the plate with a plate sealer, place the plate in a thermostat shaking incubator at 37°C, and incubate for 60 minutes at 200 ~ 300 rpm.
8. Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.
9. Color development: Add 100 µL of TMB Substrate to each well, gently shake to mix well, seal the plate with a plate sealer, and place the plate at 25°C for 10 minutes for color development reaction.
10. Assay: Add 50 µL of Stop Solution to each well and gently shake to mix well. Measure the optical density (OD) value of each well with a microplate reader at a primary wavelength of 450 nm and a reference wavelength of 630 nm.

Calculation

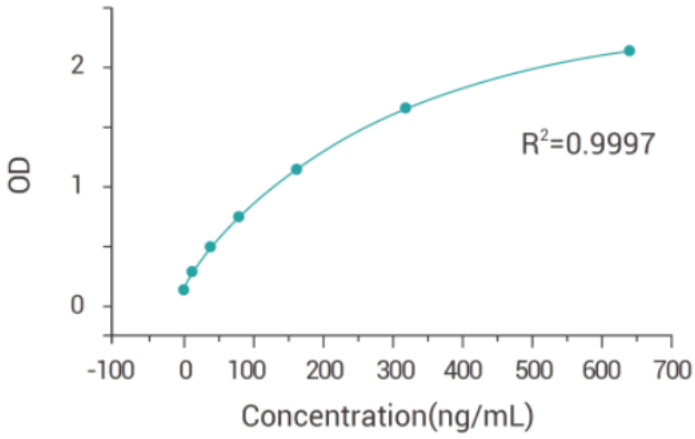
The 4-parameter fitting method is recommended for the linear fitting and calculation of the product.

1. 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

2. 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)

Typical Standard Curve



Precision

CV%≤10%, RE%≤±15%

Detection Range

20-640 ng/mL

Detection Limit

Limit of quantification: 20 ng/mL

Limit of detection: 5 ng/mL

Precautions

1. If the test samples are purified, it is usually recommended to detect with the original solution or 2-fold diluted solution. When testing for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve. Diluent should be mixed thoroughly before further analysis or dilution. Analyze each sample in duplicate to determine the correct residual RNase Inhibitor value in the original sample.
2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.
3. Before using the pre-coated strips, please equilibrate to room temperature and then open the secondary packaging. The strips not used in the test should be immediately placed back into the package and sealed, and can be stored at 4°C for one month. Other unused reagents should be packaged or covered.
4. The volumes of standard, biotin, and enzyme conjugate are all very small. Please perform rapid centrifugation before use to let liquid on the tube wall or cap gather at tube bottom.
5. Please use disposable tips during experimental operation to avoid cross contamination.
6. Please check each reagent in the kit before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.

7. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well to absorb the liquid.
8. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
9. The kit is for single use. Please use within the shelf life.

Limitations

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