Creative Diagnostics®

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**User's Manual**

# Double-stranded RNA (dsRNA, modified) ELISA kit

REF **DEIA-BZ002**

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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.

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# PRODUCT INFORMATION

## Intended Use

This kit uses double-antibody sandwich enzyme-linked immunosorbent assay to detect the residues of double-stranded RNA (dsRNA).

## General Description

mRNA has gained significant worldwide attention as a novel active ingredient in vaccines and gene therapies. The increasing demand for mRNA molecules has compelled mRNA manufacturers to quickly scale up production capacity while maintaining high mRNA quality. In vitro transcription by T7 polymerase is the standard procedure to synthesize mRNA. However, this procedure may introduce double-stranded RNA (dsRNA) contaminants from random priming of abortive transcripts, turn-around transcription, and antisense transcription. dsRNA immune activation results in the up-regulation of various pro-inflammatory cytokines, and cell death, which can lead to patient morbidity. Therefore, to improve the quality of mRNA translation, and minimize adverse effects, it is critical to carefully monitor in vitro-transcribed (IVT) mRNA products and confirm the removal of dsRNA after purification.

## Principles of Testing

This kit is based on double antibody sandwich ELISA. Microplates are coated with dsRNA monoclonal antibodies to capture dsRNA antigens in the sample. After the detection antibody is added, the HRP conjugated antibody can be added to form a complex. After washing, the substrate solution was added to develop color. The chromogenic solution turns blue under the catalysis of HRP-conjugated antibody, and finally turns yellow under the action of acid. The depth of color is positively correlated with the amount of dsRNA in the sample.

## Reagents And Materials Provided

1. Microtiter Plate (coated with dsRNA monoclonal antibodies), 1 plate, 8×12 wells
2. dsRNA (modified) Calibrator (300 ng/mL), 100 µL
3. Sample Diluent, 30 mL×2
5. HRP-Conjugated Antibody Dilution, 12 mL
6. Detection Antibody (100×), 120 µL
7. HRP-Conjugated Antibody (100×), 100 µL
8. Wash Buffer (20×), 30 mL
9. Substrate Solution, 12 mL
10. Stop Solution, 6 mL
11. Sealers, 4 sheets

## Materials Required But Not Supplied

1. Deionized or distilled water
2. Oscillator
3. Plate washer
4. Micropipettes and matching sterilized tips
5. Incubator or water bath
6. Microplate reader
7. Sample tank
8. Absorbent paper

## Storage

The kit should be stored at 2-8°C. Aavoid direct sunlight. The validity period is 12 months.

## Reagent Preparation

**1. Allow all the reagents to equilibrate to room temperature (18-28°C) prior to use. The reagents should be stored at 2-8°C after use.**

**2. Wash Buffer:** 30 mL 20× Wash Buffer+570 mL distilled or de-ionized water.

**3. dsRNA (modified) Calibrator:** The calibrator is reconstituted by pipetting the amount of sample dilution buffer. Prepare each standard in tubes by 2-fold serial dilution of the reconstituted calibrator with Sample Dilution buffer. After reconstitution the calibrator cannot be stored for repeated use.

| Volume of dsRNA (modified) Calibrator | Sample Dilution | Concentration(ng/mL) |
|---|---|---|
| 300 ng/mL Calibrator 5 µL | 995 µL | 1.5 |
| 1.5 ng/mL Calibrator 500 µL | 500 µL | 0.75 |
| 0.75 ng/mL Calibrator 500 µL | 500 µL | 0.375 |
| 0.375 ng/mL Calibrator 500 µL | 500 µL | 0.187 |
| 0.187 ng/mL Calibrator 500 µL | 500 µL | 0.093 |
| 0.093 ng/mL Calibrator 500 µL | 500 µL | 0.047 |
| 0.047 ng/mL Calibrator 500 µL | 500 µL | 0.023 |
| 500 µL Sample Dilution | Blank | 0 |

**4. 1 × Detection Antibody:** Dilute the detection antibody (100 ×) to 1 × with the Sample Diluent in the volume ratio of 1:99. The dilution volume (100 µl per well) was determined based on the number of assays.

**5. 1 × HRP-Conjugated Antibody:** Dilute the HRP-Conjugated Antibody (100 ×) to 1 × with HRP-Conjugated Antibody Dilution in the volume ratio of 1:99. The dilution volume (100 µl per well) was determined based on the number of assays.

## Assay Procedure

1. Transfer 100 µL in duplicate of calibrators and samples into appropriate wells. Do not touch the side or bottom of the wells.

**Note: When the dsRNA content in the sample to be tested cannot be determined, the sample should be diluted for multiple dilutions for detection.**

2. Cover the plate. Incubate the plate for 1 hour at 37°C.
3. 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 3b.

   d. Repeat the washing procedure 3b/3c three times.

   e. Empty the plate and gently tap on thick layer of tissues.
4. Add 100 µl of 1× detection antibody to each well using the same pipetting order as applied in step 1.
5. Cover the plate. Incubate the plate for 1 hour at 37°C.
6. Repeat the wash procedure described in step 3.
7. Add 100 µL of diluted 1× enzyme-labeled secondary antibody to each well using the same pipetting order as applied in step 1
8. Cover the plate. Incubate the tray for 1 hour at 37°C.
9. Repeat the wash procedure described in step 3.
10. Add 100 µL of Substrate Solution to each well, using the same pipetting order as applied in step 1. Cover the plate and incubate the plate for 15 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 1. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells. 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.

**Note: 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 calibrators and samples.
2. Create a standard curve using computer software. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis, as well as the corresponding concentration on the horizontal (X) axis (logarithmic scale).
3. Lowest limit of quantification (LOQ) = 47 pg/mL. Below 47 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.

## Precautions

1. Please strictly follow the instructions for the storage and usage of each component.
2. Avoid experimenting in harsh environments. Do not perform experiments in an environment containing RNase III and toti-nuclease.
3. 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. Do not open the reagent until it has come to room temperature. Shake well before use.
4. After taking out the desired ELISA plate, put the rest in a sealed bag and store at 2-8°C. Do not touch the bottom of the wells.
5. Avoid creating air bubbles when adding samples. Avoid touching the bottom of the well with the pipette to prevent scratches.
6. The parafilm is not reusable. Different batches of reagents cannot be mixed. Pipettes cannot be mixed.
7. If the concentrated washing solution crystallizes, it should be placed at 37°C until dissolved before use. When washing the plate, it should be washed thoroughly.
8. This product is for scientific research only.
9. 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.