V. 250601

Product Information & Manual

Double-stranded RNA (dsRNA) ELISA Kit (J2 based)

Enzyme-linked immunosorbent assay for quantitative detection of Double-stranded RNA

Catalogue Number C13005-K01

For Research Use Only. Not for use in diagnostic and therapeutic procedures.

RUO

96 wells

store at 2-8°C

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PRODUCT INFORMATION

Croyez ® Double-stranded RNA (dsRNA) ELISA Kit (J2 based)

1. Introduction

The Croyez Double-stranded RNA (dsRNA) ELISA Kit is a highly sensitive and quantitative immunoassay developed based on the double-antibody sandwich ELISA platform. It enables accurate detection of dsRNA contaminants commonly present in in vitro transcribed (IVT) mRNA or mRNA vaccine formulations.

Residual dsRNA is an unwanted by-product during IVT synthesis and is known to trigger innate immune responses. Therefore, monitoring and minimizing dsRNA levels is essential for ensuring mRNA product quality, safety, and translational efficiency.

This kit is specifically optimized for use in mRNA drug development, QC release testing, and process optimization, providing a robust tool for detecting trace levels of dsRNA in a variety of sample types.

2. Test principle

This Double-stranded RNA (dsRNA) ELISA Kit utilizes a sandwich ELISA method to detect dsRNA in samples. The assay features a microplate pre-coated with mouse anti-dsRNA monoclonal antibody. DsRNA in the sample binds to this solid phase, then reacts with HRP-conjugated mouse anti-dsRNA monoclonal antibody. After washing away unbound antibody, a substrate solution is added, and the reaction is measured by absorbance at 450 nm. DsRNA levels are quantified by comparing sample absorbance to a standard curve.

3. Key Features

- High specificity and sensitivity for double-stranded RNA
- Double-antibody sandwich ELISA format for quantitative results
- Broad sample compatibility: IVT RNA, synthetic mRNA, formulated mRNA vaccines
- Compatible with high-throughput workflows
- Easy-to-use protocol; no need for dsRNA purification prior to assay
- Provides reliable standard curve for accurate quantification

4. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
Double-stranded RNA (dsRNA) ELISA plate Stripwell microplate with 96 anti-Double stranded RNA monoclonal antibodies coated wells	96 wells (12 x 8 well strips)	Ready for use
Standard Double-stranded RNA (dsRNA) from buffered protein solution with preservatives	1 vials (250 µL)	Refer to the vial label for reconstitution volume. Reconstitute by adding Standard reconstitution buffer to be a stock solution of 500 ng/mL. **(see Procedure, section 2)**
Standard & Sample diluent buffer Buffered protein solution with preservatives	1 vial (12 mL)	Ready for use
HRP-antibody conjugate HRP conjugated anti- Double-stranded RNA monoclonal antibody in buffered protein solution with preservatives	1 vial (70 µL)	Dilute 200 x with HRP-antibody conjugated diluent buffer **(see Reagent preparation, section A)**
HRP-antibody conjugated diluent buffer Buffered solution with preservatives	1 vial (12 mL)	Ready for use
20 X wash buffer 20-fold concentrated solution of buffered surfactant with preservatives	1 vial (15 mL)	Dilute 20 x with distilled water **(see Reagent preparation, section B)**
TMB Chromogenic substrate (tetramethylbenzidine) for HRP	1 vial (12 mL)	Ready for use
Stop solution H_2SO_4 solution	1 vial (6 mL)	Ready for use
Microplate sealing film	2 sheet	N/A

5. Application

- Quality Control for mRNA Vaccines and Therapeutics
- Process Optimization in IVT mRNA Manufacturing
- Immunogenicity Risk Assessment

6. Materials required but not provided

(1) High quality distilled water

(2) 10 mL graduated pipettes

(3) 5 µL to 1000 µL adjustable single-channel micropipettes with disposable tips

(4) 50 µL to 300 µL adjustable multi-channel micropipettes with disposable tips

(5) Multi-channel micropipette reservoir

(6) Disposable microcentrifuge tubes

(7) Beakers, flasks, cylinders necessary for preparation of reagents

(8) Timer

(9) Magnetic stirrer

(10) Vortex mixer

(11) Washer for microplates

(12) Incubator capable of maintaining temperature at 37±1°C

(13) Stripwell microplate spectrophotometer capable of reading at 450 nm

(14) Clean paper towels

(15) Disposable gloves

(16) Discard container for bio-medical waste

7. Reagent preparation

All the working reagents should be prepared with adequate volume and discarded at the end of the day.

A. Working HRP-antibody conjugate (1 X): Dilute 1 volume of **HRP-antibody conjugate** with 199 volumes of **HRP antibody conjugated diluent buffer** and homogenize by vortex.

B. Working wash buffer (1 X): Dilute 1 volume of **20 X wash buffer** with 19 volumes of distilled water and homogenize by using a magnetic stirrer.

8. Storage and expiration date of reagents

- Before opened, all kit reagents should be kept properly at 2-8°C. Please see the box front label for expiration date.
- Once opened, the kit should be used within 2 weeks, and the remaining reagents should be immediately returned to 2-8°C after used, except the reconstituted standard, it must be stored at -80°C.
- Avoid multiple freeze-thaw cycles of the frozen standard, and if stored properly at -80°C, it should be valid for maximum 2 weeks.
- Unused strips must be stored at 2-8°C in a sealed bag containing a desiccant and should be used as soon as possible.
- All working reagents, Working HRP-antibody conjugate (1 X) and Working wash buffer (1 X), should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.

9. Precautions & warnings

In order to obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those persons who have been properly trained.
- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents (especially TMB and Stop solution, which contains H_2SO_4) contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.
- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- This product is designed for a single, one-time use only.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- Do not mix or substitute reagents with those from different lots or other sources.

- Bring all the reagents and specimens to 15-30°C prior to use.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Do not expose all the reagents to strong light during storage or incubation.
- Avoid contact of TMB with metal to prevent color development. The color of TMB should be colorless. If a blue color develops before use, indicating it is unusable, it must be discarded.
- Use disposable graduated pipettes and tips to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

10. Procedure

1. Evaluate the number of stripwell required to test the samples. Put the stripwells at room temperature (15-30°C) before use. The unused strips should be resealed in the bag and stored at 2-8°C. Each standard, blank, and sample should be assayed in duplicate.

2. Standard and sample preparation:

(1) Standard preparation (in microcentrifuge tubes):

- Aliquot and store the standards at -20°C
- Add 400 µL Standard & sample diluent buffer to 100 µL of 500 ng/mL standard to make a 100 ng/mL standard (Tube 1).
- Adding 250 µL of Standard & sample diluent buffer to 250 µL of 100 ng/mL standard to make a 50 ng/mL standard (Tube 2).
- Repeat the above procedure to make serial diluted standards (Tube 3-7).
- Tube 8 is blank which only contains Standard & sample diluent buffer.

(2) Sample preparation:

- 100 µL Sample. If the initial assay found samples contain double-stranded RNA (dsRNA) higher than the highest standard, the samples can be diluted with Standard & sample diluent buffer and then re-assay the samples.
- Add 100 µL of standards, blanks or samples into double-stranded RNA (dsRNA) ELISA stripwell microplates (see Table 1). Cover with microplate sealing film and incubate

sealed plate at 37°C for 1 hour.

- Remove the sealing film, aspirate the liquid from each well and then wash the plate three times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.
- Add 100 µL of Working HRP-antibody conjugate into each well. Cover with microplate sealing film and incubate sealed plate at 37°C for 1 hour in the dark.
- Remove the sealing film, aspirate the liquid from each well and then wash the plate six times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.
- Add 100 µL of TMB into each well. Incubate for 6 minutes at room temperature (15-30°C) in the dark.
- Add 50 µL Stop solution into each well.
- Read the absorbencies immediately at 450 nm after the Stop solution is added.

Table 1 An example of orientation of standards,blanks and samples in the stripwells microplate

	1	2	3	4
A	Standard 1 (100 ng/mL)	Standard 1 (100 ng/mL)	Sample 1	Sample 5
B	Standard 2 (50 ng/mL)	Standard 2 (50 ng/mL)	Sample 1	Sample 5
C	Standard 3 (25 ng/mL)	Standard 3 (25 ng/mL)	Sample 2	Sample 6
D	Standard 4 (12.5 ng/mL)	Standard 4 (12.5 ng/mL)	Sample 2	Sample 6
E	Standard 5 (6.25 ng/mL)	Standard 5 (6.25 ng/mL)	Sample 3	Sample 7
F	Standard 6 (3.125 ng/mL)	Standard 6 (3.125 ng/mL)	Sample 3	Sample 7
G	Standard 7 (1.562 ng/mL)	Standard 7 (1.562 ng/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

11. Internal quality control

- The average absorbance of Blank: ≦0.1
- The average absorbance of highest concentration of standard (100 ng/mL): ≧1.0

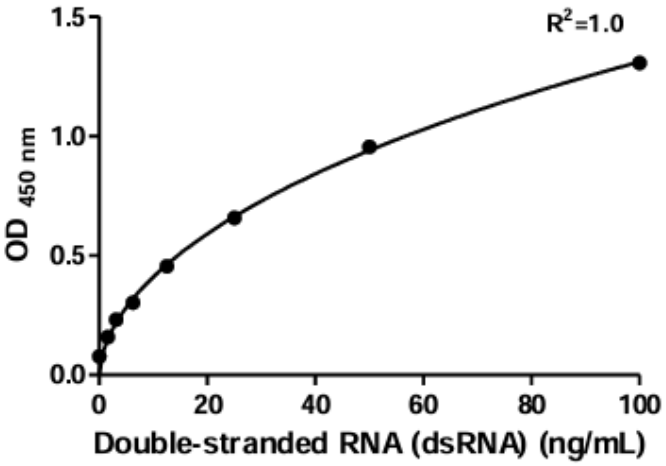
12. Calculation of results

- The standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis) using four-parameter logistic (4-PL) curve fit.
- The double-stranded RNA (dsRNA) concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 100 ng/mL, the samples should be diluted with Standard & sample diluent buffer and re-assay again.

Typical data

The following data are for demonstration only

Standard	double-stranded RNA (dsRNA) (ng/mL)	$OD_{450\ nm}$	
1	100	1.319	1.294
2	50	0.961	0.949
3	25	0.649	0.668
4	12.5	0.459	0.452
5	6.25	0.301	0.305
6	3.125	0.241	0.223
7	1.562	0.1443	0.175
Blank	0	0.072	0.085



13. Assay limitations

- Sample should be centrifuged to remove debris.

14. Performance characteristics

Sensitivity

- The limit of detection (LoD) of double-stranded RNA (dsRNA) ELISA Kit is 0.288 ng/mL.
- The limit of quantification (LoQ) of double-stranded RNA (dsRNA) ELISA Kit is 1.694 ng/mL.