RES92-EN.01

Double-Stranded RNA (dsRNA) ELISA Kit
(Enzyme-Linked Immunosorbent Assay)

Catalog Number：RES-A092

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

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Content

INTENDED USE

Double-Stranded RNA (dsRNA) ELISA Kit was developed for the detection and quantitative determination of dsRNA in samples. It is intended for research use only (RUO).

BACKGROUND

dsRNA is a byproduct of the in vitro transcription performed during the preparation of mRNA for the vaccine formulation. During this step, impurities due to errors in the transcription enzyme can cause the formation of double-stranded RNA (dsRNA). Double-stranded RNA is a marker of viral infection and is considered an impurity in mRNA vaccines.

To support the development of biologic products, ACROBiosystems developed Double-Stranded RNA (dsRNA) ELISA Kit with rigorous methodological validation, which is used evaluation the quality of biologic products in vaccine development and CMC quality control stages.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of dsRNA by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-dsRNA Monoclonal Antibody (K1). Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the HRP-Anti-dsRNA Monoclonal Antibody (J2) to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of dsRNA present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of dsRNA bound.

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PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not use reagents past their expiration date.
3. Do not mix or substitute reagents with those from other kits or other lot number kits.
4. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
5. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 T)	Format	Storage	
				Unopened	Opened
RES092-C01	Pre-coated Anti-dsRNA Monoclonal Antibody (K1) Microplate	1 plate	Solid	2-8℃	2-8℃
RES092-C02	Unmodified dsRNA (400bp) Standard	10μg×2	Powder	2-8℃	-70℃
RES092-C03	HRP-Anti-dsRNA Monoclonal Antibody (J2)	10μg×2	Powder	2-8℃, avoid light	-70℃, avoid light
RES092-C04	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
RES092-C05	Sample Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RES092-C06	HRP-Antibody (J2) Dilution Buffer	25 mL	Liquid	2-8℃	2-8℃
RES092-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RES092-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

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SRORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note:

a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

1. Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;
2. 37ºC Incubator;
3. Single or dual wavelength microplate reader with 450 nm and 630 nm filter;
4. Tubes: 1.5 mL,10 mL;
5. Timer;
6. Reagent bottle;
7. Deionized or distilled water.

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QUICK GUILD

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REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 4 μg.

Note: *Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES092-C02	Unmodified dsRNA (400bp) Standard	10μg	200 μg/mL	50 μL
RES092-C03	HRP-Anti-dsRNA Monoclonal Antibody	10μg	100 μg/mL	100 μL

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of HRP-Anti-dsRNA Monoclonal Antibody (J2) working fluid:

Dilute HRP-Anti-dsRNA Monoclonal Antibody (J2) to 0.5 μg/mL with HRP-Antibody (J2) Dilution Buffer. Please prepare it for one-time use only.

2. Preparation of Standard curve

The concentration of the reconstituted dsRNA Calibrator (RES092-C02) is 200 μg/mL，prepare (Std.-0)

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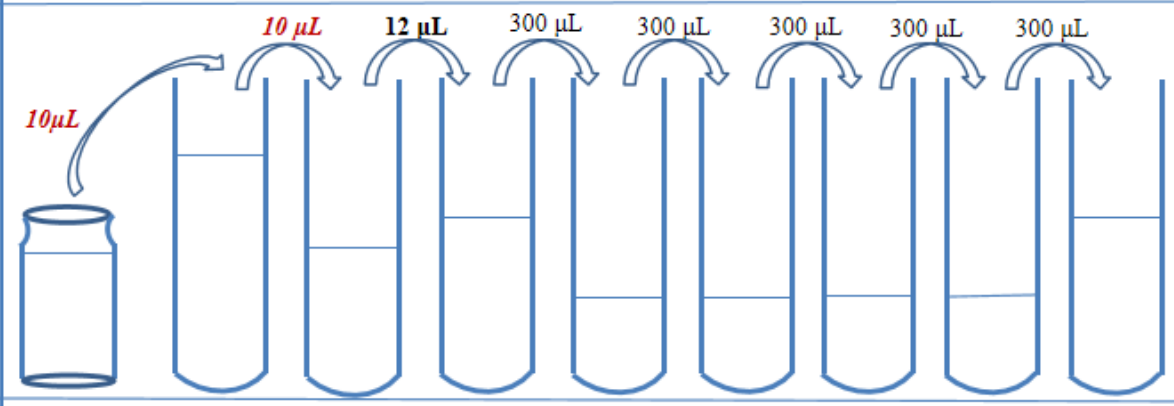
by diluting 10 µL the reconstituted dsRNA Calibrator into 990 µL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 10 µL Std.-0 into 490 µL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, Std.-1 (800 pg/mL), by diluting 12 µL Std.- 1' into 588 µL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.

Tubes/ Solution Code	Unmodified dsRNA (400bp) Standard stock solution	Std.-0	Std.-1'	**Std.-1**	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6
Operating		*10 µL*	**12 µL**	300 µL	300 µL	300 µL	300 µL	300 µL	
Solution Con.	200µg/mL	2000 ng/mL	40 ng/mL	**800 pg/mL**	400 pg/mL	200 pg/mL	100 pg/mL	50 pg/mL	25 pg/mL
Dilution Buffer Vol.		*990 µL*	*490 µL*	**588 µL**	300 µL	300 µL	300 µL	300 µL	300 µL



3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Sample Dilution Buffer.

Note:

1. *It is recommended to set double holes for samples and standard curves to be tested.*
2. *Blank is a Blank Dilution Buffer hole.*

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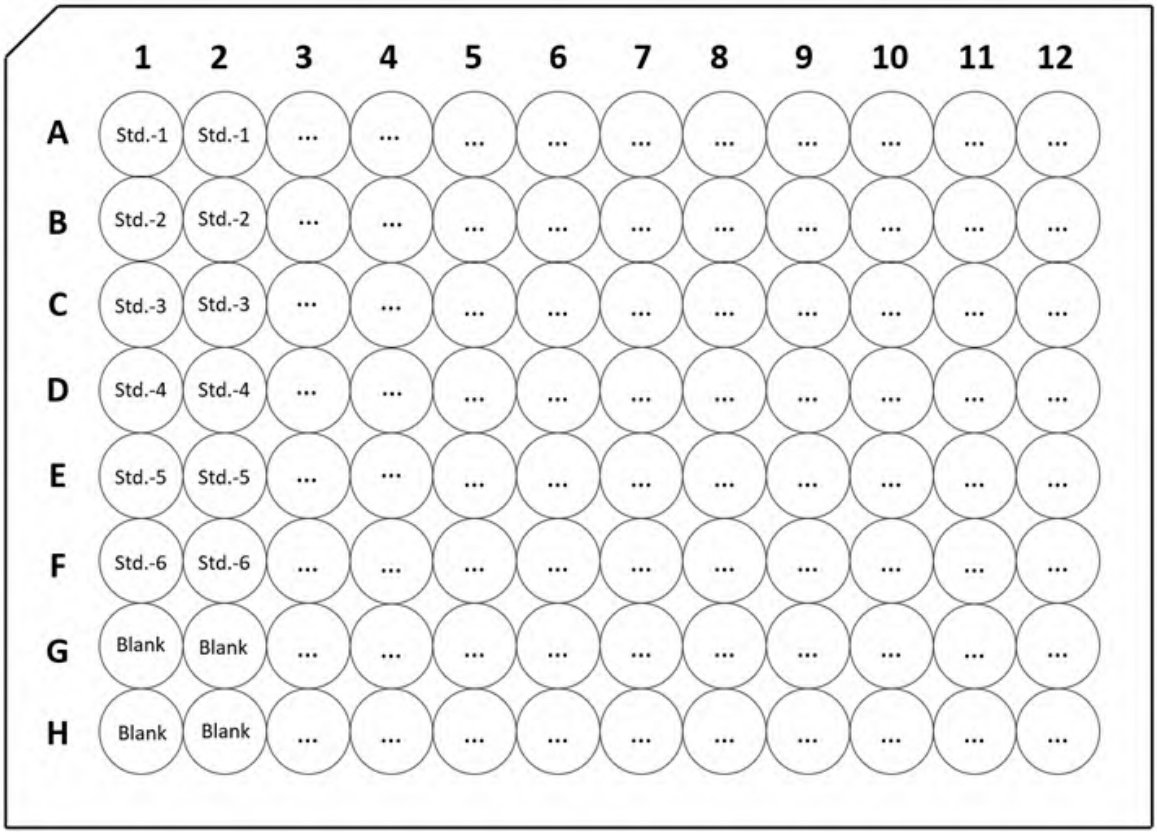

4. Incubation

Seal the plate with microplate sealing film, shake at 400 rpm, and incubate at room temperature for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add HRP-Anti-dsRNA Monoclonal Antibody (J2)

For all wells, add 100 µL HRP-Anti-dsRNA Monoclonal Antibody (J2) (dilute to 0.5 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film, shake at 400 rpm, and incubate at room temperature for 1.0 hour.

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8. Washing

Repeat step 5.

9. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

10. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

11. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note*: To reduce the background noise, subtract the value read at* OD_{450nm} *with the value read at* $OD_{630\ nm}$.

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. Detection range: 25 pg/mL-800 pg/mL. If the OD value of the sample to be tested is higher than 800 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 25 pg/mL, the sample should be reported.

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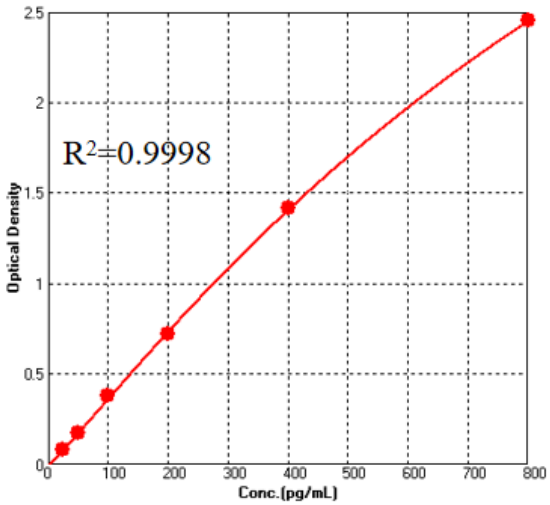
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
800	2.502	2.448	2.475	2.451
400	1.454	1.424	1.439	1.415
200	0.755	0.735	0.745	0.721
100	0.405	0.403	0.404	0.380
50	0.203	0.192	0.198	0.173
25	0.106	0.102	0.104	0.080
0	0.024	0.025	0.025	/



SENSITIVITY

The minimum detectable concentration of dsRNA is 8.988 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	661.110	164.505	66.802	659.921	163.056	65.590
SD	40.762	8.868	3.295	13.344	4.376	2.396
CV (%)	6.2	5.4	4.9	2.0	2.7	3.7

***Note**: The example data is for reference only.*

RECOVERY

Three samples with different concentrations were tested to calculate the recovery rate.

Sample(n=5)	Average Recovery %	Range %
High	105.5	94.6-113.8
Middle	95.7	88.5-100.3
Low	95.6	84.1-109.0.

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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