ELISA PRODUCT INFORMATION & MANUAL

Multi-Species dsRNA ELISA Kit (Colorimetric)

NBP3-11367

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Table of Contents

Intended Use	3
Storage of Kit Components	3
Background and Principle of the Assay	3
Materials Provided	4
Materials Required but Not Provided	5
Preparation of Reagents	5
Assay Protocol	5-8
Precautions and Recommendations	8

Intended Use

The Novus Biologicals Multi-Species dsRNA ELISA Kit (Colorimetric) can be used to detect viral dsRNAs or large natural or synthetic dsRNAs of non-viral origin in nucleic acid extracts, as well as to detect the presence of undesired dsRNA molecules in artificially synthesized (m)RNA preparations. By using serial dilutions of the Poly (I:C) dsRNA standard (included in the kit) for calibration, quantitative estimates can be made.

This assay is for research use only and not for use in diagnostic or therapeutic procedures.

Storage of Kit Components

The Multi-Species dsRNA ELISA Kit (Colorimetric) components are shipped on cold packs. Upon receipt, store entire kit at -20ºC. Once the kit is thawed, you may keep it at 4ºC for 5 days. For long-term storage, it is recommended to aliquot and freeze the antibodies and dsRNA component at –20ºC.

Background and Principle of the Assay

Based on the use of two double-stranded RNA (dsRNA)-specific monoclonal antibodies the dsRNA Detection Kit allows sensitive and selective detection of dsRNA molecules (larger than 30-40 bp), independent of their nucleotide composition and sequence. The detection is highly specific: dsRNA can be detected in nucleic acid extracts in the presence of 1.000-10.000-fold excess of other nucleic acids. This assay works on the sandwich-ELISA principle and uses the J2 (IgG2a) mouse monoclonal antibody to dsRNA as a catcher antibody. The monoclonal antibody K2 (IgM) is used as the detector antibody.

Materials Provided

The Multi-Species dsRNA ELISA Kit (Colorimetric) is provided in 200 and 500 test size. Volumes listed below are for the 200 test kit followed by the 500 test kit.

	Component name and information	200 test kit	500 test kit
1.	Coating antibody	63 µl	156 µl
2.	Poly (I:C) dsRNA as positive control in RNAse/DNAse-free, sterile STE buffer. The concentration is 1µg/µl. (Store at -20°C or -80°C)	4 µl	10 µl
3.	dsRNA-specific detecting antibody (in RPMI + 5% FBS)	22 ml	55 ml
4.	HRP-conjugated F(ab')2 Fragment of goat-anti mouse secondary antibody	3 µl	5 µl
5.	TMB substrate solution (store at +4°C, keep in dark)	22 ml	55 ml

Materials Required But Not Provided

1. 2 ELISA plates (96 wells; e.g. Nunc Immunoplate F96 Maxisorp or Costar cat nr 2595)
2. Microtiter plate reader spectrophotometer with wavelength capability at 450 nm.
3. Single channel pipettes 10 µl and 200 µl.
4. Multichannel pipettes 200 µl or squirt bottle.
5. Antigen (standard and sample) diluent (STE Buffer: 0.1 M NaCl, 1 mM EDTA, 50 mM Tris-HCl, pH 7.0)
6. Washing Buffer (PBS + 0.5% Tween 20; PBS: 10 mM Pi-buffer, pH 7.2, 0.15 M NaCl).
7. Secondary antibody dilution buffer (PBS+1% BSA).
8. Blocking buffer (PBS containing 1% BSA and 0.2% NaN3)
9. Optional storage buffer (PBS containing 0.2% NaN3)
10. Incubator allowing incubation at 37 °C.
11. 2 M H2SO4.

Preparation of Reagents

1. Use DEPC treated MilliQ water to prepare STE (when applicable for your own sample preparation)
2. Sterilize PBS and STE by autoclaving or filter through a 0.2 micron filter
3. Prepare PBS+1% BSA, ELISA washing buffer and blocking buffer.
4. Prepare storage buffer if needed.

Assay Protocol

1. Coating the plates
    a. For two plates, add 63 µl of Coating antibody into 21 ml PBS, mix well and immediately distribute 100 µl/well in 2 ELISA plates. For five plates, add 156 µl of Coating antibody into 52 ml PBS, mix well and immediately distribute 100 µl/well in 5 ELISA plates.

b. Cover the plates and incubate them overnight at 4 °C.
c. Discard contents of wells into waste. Add 100 µl/well 1% BSA in PBS + 0.2% NaN3 to each well and incubate at 37 °C for 2 h to saturate any remaining free binding sites on the plate.
d. Discard the solution and wash plates 3 times with PBS + 0.5% Tween 20.
e. The plates can then be used directly or stored. For storage fill the wells with 200 µl/well PBS containing 0.2% sodium azide.

2. Preparation of the Poly (I:C) dsRNA Positive Control.
   - Prepare 1:3 serial dilutions from Poly (I:C) dsRNA positive control by using RNAse/DNAse-free, sterile STE buffer.
   - The dilution series of the dsRNA standard should be in the range of expected dsRNA concentration of your sample.
   - We propose starting with 30 ng dsRNA/well as the highest concentration and diluting down to below 0.01 ng dsRNA/well.
   - Dilutions should be freshly made for each assay.
3. Preparations of Sample
   - Prepare dilutions of your sample in STE (when necessary).
   - Cap and vortex all diluted standards and samples.
4. Wash Step
   - Remove the plastic foil from the ELISA plate
   - Discard contents of wells into waste. Wash plate 4 times with PBS + 0.5 % Tween 20 adding 250 µl washing solution/well. Discard the solution.
   - Do not allow wells to dry before adding the next solution.
5. Addition of the Antigen

- Transfer 100 µl antigen or diluted standard to duplicated wells in the plate.
- Cover and Incubate 1 hour at 37 °C.

6. Wash Step
   - Discard contents of wells into waste. Wash plate 4 times with PBS + 0.5 % Tween 20 adding 250 µl washing solution/well.
   - Do not allow wells to dry before adding the next solution.
7. Detector Antibody Addition
   - Pipette 100 µl undiluted dsRNA-specific detecting antibody into all wells.
   - Incubate 1 hour at 37 °C.
8. Dilute secondary antibody
   - During the incubation (step 5) dilute HRP-conjugated secondary antibody by pipetting
     1.3 µl into 21 ml PBS + 1% BSA (no azide!) for 200 tests
     or 3.25 µl HRP-conjugated secondary antibody into 52 ml PBS + 1% BSA (no azide!) for 500 tests.
9. Wash Step
   - Discard contents of wells into waste. Wash plate 4 times with PBS + 0.5 % Tween 20 adding 250 µl washing solution/well.
   - Do not allow wells to dry before adding the next solution
10. Secondary Antibody Addition
    - Add 100 µl diluted HRP-conjugated secondary antibody into each well.
    - Incubate 1 hour at 37 °C.
11. Final Wash Step
    - Discard contents of wells into waste. Wash plate 4 times with PBS + 0.5 % Tween 20 adding 250 µl washing solution/well.
    - Do not allow wells to dry before adding the next solution
    - Take care to remove all washing fluid after the last wash.
12. Development
    - Add 100 µl of TMB substrate solution into each well. Incubate for 5-60 minutes at room temperature in the dark.

13. Stop
    - When the absorbance has reached the optimum level stop reaction by adding 100 µl of 2M H_2SO_4 to all wells.
14. Read
    - Read absorbance at 450 nm, blanking on the zero standard.

Precautions and Recommendations

1. All standards and samples should be assayed at least in duplicate.
2. Use clean, RNase-free micro-centrifuge tubes with cap.
3. Do not use buffers which contain NaN_3 as it will interfere with the final detection step.
4. Do not expose reagents to excessive light.
5. Wear disposable gloves and eye protection.
6. Do not use the kit beyond the expiration date.
7. Do not mix reagents from different kits.
8. Do not mouth pipette or ingest any of the reagents.
9. The buffers and reagents used in this kit contain anti-microbial and anti-fungal reagents. Care should be taken to prevent direct contact with these products.
10. Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
11. Human samples may be contaminated with infectious agents. Do not ingest, expose to open wounds, or breathe aerosols. Dispose of samples properly.
12. After completion of step 1: Cell Plating the plates can be stored without any loss of activity for one month. To store wrap plates in plastic foil and store them refrigerated at 4 °C. When stored plates are used, they must be thoroughly washed with PBS to remove all traces of NaN_3.