Double-stranded RNA (dsRNA) ELISA kit (K1 based)

Features

- Highly sensitive detection of dsRNA
- Detects plant and animal viruses
- Distinguishes bacterial from viral pathogens

Ordering Information

Catalog Number + (size)
10623002 (200 tests)
10623005 (500 tests)

Format
ELISA Kit

Species Reactivity
Ubiquitous

Company Information
Exalpha Biologicals, Inc.
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Other Nucleic Acid Detection Kits & Reagents Available from Exalpha Biologicals

Double-stranded RNA Detection Kit (J2 based)
10613002 (200 Tests)
10613005 (500 Tests)

Mouse anti double-stranded RNA (J2, J5 and K1) Set
10040200 (3x100 µg)

DNA Fragmentation Detection Kit
X2044K1 (30 Tests)
X2044K2 (60 Tests)

BrdU Cell Proliferation Assay Kit
X1327K1 (200 Tests)
X1327K2 (1000 Tests)

BrdU Chemiluminescent Cell Proliferation Assay Kit
X1623K1 (200 Tests)
X1623K2 (1000 Tests)

BrdU Immunohistochemistry Kit
X1545K.1 (50 Sections)

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Intended Use

The Exalpha Biologicals, Inc. Double-stranded RNA (dsRNA) ELISA kit (K1 based) 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. The Poly(I:C) dsRNA positive control included in this sandwich ELISA kit is only intended to be used as a positive control to see if the ELISA has been executed correctly and that the test shows a linear relationship between the amount of dsRNA and the read out for the OD450. It is not intended to be used as a quantitative standard for other dsRNA preparations since the anti dsRNA antibodies used in this kit may exhibit a different degree of reactivity with different dsRNAs obtained from synthetic or natural sources. The only proper standard for each specific application is the purified dsRNA under investigation.

Also, the Poly(I:C) control dsRNA can be used for comparison of the outcomes of incubations with the same kit lot at different time points or for comparison of the outcomes of incubations with different kit lots.

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

Storage of Kit Components

Exalpha's Double-stranded RNA (dsRNA) ELISA Kit 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 K1 (IgG2a) mouse monoclonal antibody to dsRNA as a catcher antibody. The monoclonal antibody K2 (IgM) is used as the detector antibody.

Over the past decade our double-stranded RNA (dsRNA) antibodies have been used extensively to detect and characterize plant and animal viruses with dsRNA genomes or intermediates. In addition, the anti-dsRNA antibodies can be used as a tool to detect pathogens, including detection in paraffin-embedded fixed tissue samples (Richardson et al. 2010).

The K1 monoclonal antibody recognizes dsRNA with similar affinity to our widely used J2 antibody. It can be used for the histological and cytological detection of dsRNA in cells and tissues.

It has proven especially useful as an alternative to J2 to resolve cross-reactions and/or remove unwanted background, in those rare experimental setups where J2 did not provide satisfactory results.

K1 can be used to detect dsRNA intermediates of viruses as diverse as Hepatitis virus, Theiler's murine encephalomyelitis virus or Japanese encephalitis virus. It has been for the detection of dsRNA in cultured cells and in fixed paraffin-embedded histological samples (see publications).

If poly(I):(C) needs to be detected we recommend using K1 rather than J2 because K1 has a much higher affinity for this synthetic polyribonucleotide (see Schönborn et al. 1991).
K1 has been used successfully in immunofluorescence microscopy, in flow cytometry (FACS) and in immunocapture methods (such as dot-blot and ELISA).

References

S. J. Richardson et al. J Clin Virol. (2010) 49(3); 180.
J. Schönborn et al. Nucleic Acids Res.(1991)19, 2993.

Materials Provided

Double-stranded RNA (dsRNA) ELISA kit (K1 based) is provided in 200, 500 test size. Volumes listed below are for the 200 test kit followed by the 500 test kit.

	Component name and information	Part #	200 test kit	500 test kit
1.	dsRNA-specific coating antibody K1	J0174	63 µl	156 µl
2.	Poly (I:C) dsRNA as positive dsRNA control in RNAse/DNAse-free, sterile STE buffer. The concentration is 1µg/µl. (Store at -20°C or -80°C)	J0171	4 µl	10 µl
3.	dsRNA-specific detector antibody (in RPMI + 5% FBS)	J0172	22 ml	55 ml
4.	HRP-conjugated goat-anti mouse secondary antibody	J0173	3 µl	5 µl
5.	TMB substrate (store at +4°C, keep in dark)	J0005	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 (control and sample) diluent (STE Buffer: Bioworld Cat# 10530010-1; Biotechnology Grade, 10 mm Tris, 1 mm EDTA, 0.1 M NaCl, pH 8.0+/-0.1)
6. Washing Buffer (PBS + 0.5% Tween 20; Composition of PBS: 8.1 mM Na_2HPO_4, 137 mM NaCl, 2.68 mM KCl, 1.47 mM KH_2PO_4, pH 7.0-7.5).
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 J0174 into 21 ml PBS, mix well and immediately distribute 100 µl/well in 2 ELISA plates. For five plates, add 156 µl of J0174

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 one week. For storage fill the wells with 200 µl/well PBS containing 0.2% sodium azide. Cover plate with plastic foil and store at 4°C.

2. Preparation of the Positive dsRNA control.
   - Prepare 1:3 serial dilutions from J0171 by using RNAse/DNAse-free, sterile STE buffer.
   - The dilution series of the dsRNA control 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 control 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 J0172 into all wells.
   - Incubate 1 hour at 37 °C.
8. Dilute secondary antibody
   - During the incubation (step 5) dilute J0173 by pipetting 1.3 µl into 21 ml PBS + 1% BSA (no azide!) for 200 tests or 3.25 µl J0173 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 J0173 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 J0005 into each well.
    - Incubate for 5-60 minutes at room temperature in the dark.
13. Stop
    - When the absorbance has reached the optimum level and 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.

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Precautions and Recommendations

1. All controls 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 NaN3 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: (Coating the plates) the plates can be stored without any loss of activity for one week. 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 NaN3.

Ordering information

Catalog Number	Size
10623002	200 tests
10623005	500 tests

Contact Information

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