

Scicons Double-stranded RNA (dsRNA) ELISA kit (J2 based)

Cat #: orb1972246 (manual)

Clone	J2/K2
Isotype	IgG2a kappa/IgM kappa
Product Type	ELISA Kit
Units	Reagents for 200 tests
Host	Mouse
Application	ELISA

Background

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. 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 diagnostic tool to detect pathogens, including detection in paraffin-embedded fixed tissue samples (Richardson et al. 2010). The J2 anti-dsRNA IgG2a monoclonal antibody has become the gold standard in dsRNA detection. It was used initially for the study of plant viruses, but since the seminal paper of Weber et al. in 2006, where J2 was used to show that all the positive strand RNA viruses tested produced copious amounts of dsRNA in infected cells, this antibody has been used extensively in a wide range of systems, as documented in over 200 scientific publications. J2 can be used to detect dsRNA intermediates of viruses as diverse as Hepatitis C virus, Dengue virus, rhinovirus, Chikungunya virus, Rabies virus, Polio virus, Classic swine fever virus, Brome mosaic virus and many more in cultured cells and also in fixed paraffin-embedded histological samples. J2 has been used to elucidate how anti-viral responses are initiated, what counter-strategies viruses have adopted to avoid them, and to explore the viral life cycle by enabling ultrastructural localization studies of viral nucleic acid replication sites (Welsch et al., 2009 & Knoops et al., 2011). J2 has also been recommended as a diagnostic tool to detect whether an unknown pathogen is bacterial or viral in nature (Richardson et al., 2010). Recently J2 has also been used to monitor the removal of dsRNA from in vitro synthesised mRNA preparations that may have potential use in gene therapy (Kariko et al., 2011). J2 has been used successfully in various immunocapture methods, such as ELISA.

Synonyms: dsRNA ELISA kit

Source

The two dsRNA antibodies used in this kit were produced as follows: Female DBA/2 mice were injected intraperitoneally with a mixture of 50 ug L-dsRNA and 75 ug methylated bovine serum albumin, emulsified in complete Freund's adjuvant. After several boosts spleen cells were fused with Sp2/0-Ag14 myeloma cells to generate the hybridoma clones J2 and K2.

Product

Double-stranded RNA (dsRNA) ELISA kit containing the following reagents for 200 tests (2x96 wells):

- 1 vial of coating antibody (store at -20°C)
- 1 vial of Poly(I:C) dsRNA as positive control (store at -20°C)
- 1 vial of dsRNA-specific detecting antibody (in RPMI + 5% FBS, store at +4 °C or, preferably aliquoted at -20 °C)
- 1 vial of HRP-conjugated F(ab')₂ Fragment of goat-anti mouse secondary antibody (store at +4 °C or aliquoted at -20 °C)
- 1 vial of TMB substrate solution (store at +4°C, keep in dark)

Applications

We recommend using the kit 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. Serial dilutions of the Poly(I:C) dsRNA standard (included in the kit) can be used as a positive control.

For the exact detection protocol, we refer to the kit manual that can be downloaded from our website.

Caution: The Poly(I:C) dsRNA positive control included in this sandwich ELISA kit is not intended to be used as a quantitative standard for other dsRNA preparations. The anti dsRNA antibodies J2 and/or K2 used in this kit may exhibit a different degree of reactivity with different dsRNAs obtained from synthetic or natural sources. It is therefore 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 example the OD450, in the 4-parameter analysis. It cannot be used to determine the concentration of a different type of dsRNA. The only proper standard for each specific application is the purified version of the 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/antibody lots.

Storage

Upon receipt, store entire kit at -20°C. Once the kit is thawed, you may keep it at 4°C for 5 days. For long-term storage, it is recommended to aliquot and freeze the antibody and dsRNA components at -20°C.

Shipping Conditions: The Double-stranded RNA (dsRNA) ELISA kit components are shipped on blue ice.

Caution

This product is intended FOR RESEARCH USE ONLY, and FOR TESTS IN VITRO, not for use in diagnostic or therapeutic procedures involving humans or animals. It may contain hazardous ingredients. Please refer to the Safety Data Sheets (SDS) for additional information and proper handling procedures. Dispose product remainders according to local regulations.