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

Catalog number: **10613005**

Clone	J2/K2
Isotype	IgG2a kappa/IgM kappa
Product Type	ELISA Kit
Units	Reagents for 500 tests
Host	Mouse
Species Reactivity	General
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 characterise 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 cylce by enabling ultrastructiural localisation 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 unkown 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 synthethised 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 intraperitonially 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-Agl4 myeloma cells to generate the hybridoma clones J2 and K2.

Product
Double-stranded RNA (dsRNA) ELISA kit containing the following reagents for 500 tests (5x96 wells): • 1 vial of coating antibody (store at -20 °C) • 1 vial of Poly(I:C) dsRNA a positive control (store at -20 °C) • 1 vial of dsRNA-specific detecting antibody (in RPMI + 5% FBS, store at +4 °C or, preferably, at -20 °C) • 1 vial of HRP-conjugated F(ab')2 Fragment of goat-anti mouse secondary antibody (store at +4 °C or 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 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 the OD450.

Storage
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 antibody and dsRNA components at –20ºC.

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.This datasheet is as accurate as reasonably achievable, but Exalpha Biologicals accepts no liability for any inaccuracies or omissions in this information.

References

1) F. Weber, V. Wagner, S. B. Rasmussen, R. Hartmann, S. R. Paludan. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. J Virol (2006), 80(10):5059-64. doi: 10.1128/JVI.80.10.5059-5064.2006. 2) S. Welsch, S. Miller, I. Romero-Brey, A. Merz, C. K. E. Bleck, P. Walther, S. D. Fuller, C. Antony, J. Krijnse-Locker, R. Bartenschlager. Composition and Three-Dimensional Architecture of the Dengue Virus Replication and Assembly Sites. Cell Host & Microbe (2009) 5(4); 365-375. doi.org/10.1016/j.chom.2009.03.007. 3) K. Knoops , M. Bárcena, R. W. Limpens, A. J. Koster, A. M. Mommaas, E. J. Snijder. Ultrastructural characterization of arterivirus replication structures: reshaping the endoplasmic reticulum to accommodate viral RNA synthesis. J Virol. (2012) 86(5); 2474-2487. doi:10.1128/JVI.06677-11. 4) S. J. Richardson, A. Willcox, D. A. Hilton, S. Tauriainen, H. Hyoty, A. J. Bone, A. K. Foulis, N. G. Morgan. Use of antisera directed against dsRNA to detect viral infections in formalin-fixed paraffin-embedded tissue. J Clin Virol. (2010) 49(3); 180-5. doi: 10.1016/j.jcv.2010.07.015. 5) K. Karikó, H. Muramatsu, J. Ludwig, D. Weissman, Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA, Nucleic Acids Research (2011) 39(21); e142, https://doi.org/10.1093/nar/gkr695. 6) Schönborn, J., Oberstrass, J., Breyel, E., Tittgen, J., Schumacher, J. and Lukacs, N. (1991) Monoclonal antibodies to double-stranded RNA as probes of RNA structure in crude nucleic acid extracts. Nucleic Acids Res.19, 2993-3000. 7) Lukacs, N. (1994) Detection of virus infection in plants and differentiation between coexisting viruses by monoclonal antibodies to double-stranded RNA. J. Virol. Methods 47, 255-272. 8) Lukacs, N. (1997) Detection of sense:antisense duplexes by structurespecific anti-RNA antibodies. In: Antisense Technology. A Practical Approach, C. Lichtenstein and W. Nellen (eds), pp. 281-295. IRL Press, Oxford. Recent publication: Tirosh Shapira, I. Abrrey Monreal, Sébastien P Dion, Mason Jager, Antoine Désilets, Andrea D Olmstead, Thierry Vandal, David W Buchholz, Brian Imbiakha, Guang Gao, Aaleigha Chin, William D Rees, Theodore Steiner, Ivan Robert Nabi, Eric Marsault, Julie Sahler, Avery August, Gerlinde Van de Walle, Gary R Whittaker, Pierre-Luc Boudreault, Hector C Aguilar, Richard Leduc, François Jean. A novel highly potent inhibitor of TMPRSS2-like proteases blocks SARS-CoV-2 variants of concern and is broadly protective against infection and mortality in mice. bioRxiv 2021.05.03.442520; doi: https://doi.org/10.1101/2021.05.03.442520 https://biorxiv.org/cgi/content/short/2021.05.03.442520v1

Safety Datasheet(s) for this product:
EA_10613002-5_SDS

Kit Manual
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