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Product datasheet

Mouse anti double-stranded RNA (J5)

nordicmubio.com/products/mouse-anti-double-stranded-rna-j5/10040500

Catalog number: **10040500**

Clone	J5
Isotype	IgG2b kappa
Product Type	Monoclonal Antibody
Units	500 µg
Host	Mouse
Application	ELISA Immunoblotting Immunocytochemistry Immunohistochemistry

Background
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 J5 IgG2b antibody recognizes dsRNA with very similar affinity and specificity to our J2 antibody (see Schonborn et al., 1991), but has a different isotype – thus allowing more flexibility for the simultaneous detection of dsRNA with other markers, particularly in immunofluorescence microscopy, and has been used to detect replicative intermediates of the fish virus Infectious Pancreatic Necrosis Virus (IPNV) (Levican-Asenjo et al., 2019) or of ECMV in Vero cells. The J5 antibody can detect all tested forms of dsRNA, including poly(A):poly(U), poly(I):poly(C) and dsRNA from viruses such as Dengue Virus, Encephalomyocarditis Virus, Vaccinia Virus, Reovirus or Cucumber Mosaic Virus. Similarly to our other antibodies dsRNA-binding of J5 is sequence-independent, as long as the length of the dsRNA exceeds 40nt. The antibody does not react with ssRNA, ssDNA or dsDNA. J5 has been tested successfully in nucleic acid ELISA, immunoblotting and immunofluorescence microscopy.

Synonyms: Mouse anti dsRNA

Source
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 clone.

Product
Mouse monoclonal antibody J5 recognises double-stranded RNA (dsRNA) provided that the length of the helix is greater than or equal to 40 bp. dsRNA-recognition is independent of the sequence and nucleotide composition of the antigen. All naturally occurring dsRNAs investigated up to now (40-50 species) as well as poly(I).poly(C) and poly(A).poly(U) have been recognised by J5, although in some assays its affinity to poly(I).poly(C) is about 10 times lower than that to other dsRNA antigens

Formulation: The lyophilised sample should be reconstituted with 500 µl sterile distilled water. The mAb will then be in PBS without any stabilisers or preservatives at a concentration of 1 mgr/ml. As a result of the lyophilisation procedure, the reconstituted antibody may contain small amounts of denatured protein in the form of aggregates that may interfere with some applications such as immunohistochemistry (e.g. by giving high backgrounds). We therefore highly recommend centrifuging (microcentrifuge) the reconstituted antibody before use and using the supernatant.

Purification Method: Affinity chromatography on Protein A-agarose.

Purity: Gel electrophoretically pure IgG antibody.

Concentration: Concentration after reconstitution: 1.00 mg/ml as determined by A280 nm (A280 nm = 1.47 corresponds to 1 mg/ml antibod

Applications
Mouse monoclonal antibody J5 can be used for ELISA, dsRNA-immunoblotting, immunoaffinity chromatography and in certain systems also for immunohistochemistry (see references). The optimum working dilution of the antibody for any specific application should be established by titration Please note that nucleic acid separation prior to dsRNA-immunoblotting must be carried out by polyacrylamide gel electrophoresis, because the sensitivity of detection is considerably lower after blotting from agarose gels. Not for use for clinical purposes. For in vitro use only.

Storage
After reconstitution antibodies should be aliquoted and stored at -20 °C or -70°C. After adding 10 mM sodium azide undiluted antibody can also be stored at +4 °C for a short period of time. For long term storage the mAb should be kept frozen. Repeated freezing/thawing cycles should be avoided. When kept lyophilized the product will remain stable for 10 years at -20 °C or -70°C.

Shipping Conditions: Ship at ambient temperature.

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) 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. 2) 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. 3) 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. 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) Levicán-Asenjo J, Soto-Rifo R, Aguayo F, Gaggero A, Leon O. Salmon cells SHK-1 internalize infectious pancreatic necrosis virus by macropinocytosis. J Fish Dis. 2019 Jul;42(7):1035-1046. doi: 10.1111/jfd.13009.

Figure 1. J5 detects dsRNA in Vero cells infected with ECMV. dsRNA is visualized with different dilutions of J5 and is not detectable in mock infected cells. Cells were fixed with 3% PFA for 30min at 2-8°C, permeabilized with 0.5% Triton-X 100 for 5min at room temperature and J5 was detected with Cy3-labelled goat anti-mouse secondary antibody. red: dsRNA, blue: DAPI nuclear stain. Images courtesy of Friedemann Weber, Justus-Liebig-University Giessen, Germany.