



Mouse anti double-stranded RNA (J2, J5 and K1) Comparison Set

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Catalog number: **10050100**

Clone	J2, J5 and K1
Isotype	IgG2a, IgG2b, IgG2a
Product Type	Monoclonal Antibody
Units	3 x 100 µg
Host	Mouse
Application	Dot blot ELISA Immuno-affinity-chromatography 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 K1 monoclonal antibody recognises 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 highly using K1 rather than J2 because K1 has a much higher affinity for this synthetic polyribonucleotide (see Schönborn et al. 1991, Fig. 2). K1 has been used successfully in immunofluorescence microscopy, in flow cytometry (FACS) and in immunocapture methods (such as dot-blot and ELISA). 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) (Levicán-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: Anti-dsRNA mAb Comparison Set

Source

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.

Product

Mouse monoclonal antibody J2 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 J2, although in some assays its affinity to poly(I).poly(C) is about 10 times lower than that to other dsRNA antigens. 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. The mAb K1 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 K1. As described by Schönborn et al. K1 shows higher affinity to poly(I).poly(C) than to the other dsRNA antigens, although the difference of apparent binding constants may vary under different experimental conditions.

Formulation: The lyophilised samples should each be reconstituted with 100 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 antibody).

Applications

Mouse monoclonal antibodies J2, J5 and K1 can be used for ELISA, dsRNA-immunoblotting, immunoaffinity chromatography and in certain systems also for immunohistochemistry (see references). The optimum working dilution of each 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

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