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Product Information

Monoclonal Anti-Rabbit Immunoglobulins-Alkaline Phosphatase, Clone RG-16 produced in mouse, purified immunoglobulin

Catalog Number A2306

Product Description

Monoclonal Anti-Rabbit Immunoglobulins (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Purified rabbit IgG was used as the immunogen. The isotype is determined using a double diffusion immunoassay and Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The immunoglobulin fraction of the ascites fluid containing anti-rabbit immunoglobulins is conjugated to alkaline phosphatase by protein cross-linking with 0.2% glutaraldehyde.

Rabbit antibodies against many analytes are in wide use as primary antibodies in various assay techniques, both in research and clinical applications. Secondary antibodies often lack species specificity for the primary rabbit immunoglobulins. In many instances, such antibodies also recognize non-related immunoglobulins that appear in the preparation being tested resulting in increased levels of background staining and false positives. To resolve this, extensive adsorbing steps must by incorporated into the manufacturing process. Monoclonal Anti-Rabbit Immunoglobulins-Alkaline Phosphatase, which does not recognize human or many other species immunoglobulins, can serve as an essential tool especially when used as a secondary reagent in immunohistochemistry.

Reagent

Solution in 0.05 M Tris buffer, pH 8.0, containing 1 mM MgCl₂, 1% BSA, 50% glycerol, and 15 mM sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store at 2-8 °C.

Specificity

The antibody is specific for an epitope on the heavy chain of rabbit IgG, IgA, and IgM. In an immunoblot of denatured, non-reduced rabbit immunoglobulins the product stains bands at the intact whole molecule and at the heavy chains. Reduction of rabbit immunoglobulins destroys the epitope. In ELISA, the product shows no cross reaction with human serum or tissue preparations, nor with human IgA, IgG, or IgM. No cross reaction is observed with IgG from the following species: bovine, cat, chicken, dog, goat, guinea pig, horse, pig, rat, sheep or turkey.

Product Profile

Direct ELISA: minimum titer 1:40,000 Titer is defined as the dilution of conjugate sufficient to give a change in absorbance of 1.0 at 405 nm after 30 minutes of substrate conversion at 25 $^{\circ}\text{C}.^1$ Microtiter plates are coated with purified rabbit IgG at a concentration of 5 $\mu\text{g/mL}$ in 0.05 M carbonate-bicarbonate buffer, pH 9.6. Carbonate-Bicarbonate Buffer capsules are available as Catalog Number C3041.

Substrate: *p*-Nitrophenyl Phosphate (pNPP), Catalog Number N2765, 1.0 mg/mL in 10% diethanolamine buffer, pH 9.8, containing 0.5 mM MgCl₂.

Immunoblotting: a working dilution of 1:160.000 - 1:320,000 is determined using immunoblot assay detecting ß-Actin in total cell extract of HeLa cells (5-10 ug per well)

Immunohistochemistry: a minimum antibody dilution of 1:160 was determined by an indirect assay using formalin-fixed, paraffin-embedded human tonsil and Anti-Human IgG, Catalog Number I8635, as the primary antibody.

Note: Working dilutions should be determined by titration assay. Due to differences in assay systems, these titers may not reflect the user's actual working dilution.

Reference

1. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).

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