

Product No. A-4312

Lot 096H8854

Anti-Mouse IgG (whole molecule)

Alkaline Phosphatase Conjugate

Antibody developed in Rabbit

Affinity Isolated Antigen Specific Antibody

Antiserum is developed in rabbit using purified mouse IgG as the immunogen. Affinity isolated antigen specific antibody is obtained from rabbit antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to mouse IgG. Rabbit anti-mouse IgG is conjugated to alkaline phosphatase by protein cross linking with 0.2% glutaraldehyde.¹ The conjugate is provided as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1 mM MgCl₂, 50% glycerol, and 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

Specificity of the antiserum is determined by immunoelectrophoresis (IEP) and Ouchterlony Double Diffusion (ODD) assays, prior to conjugation. By IEP, the antiserum reacts specifically with normal mouse serum and mouse IgG. By ODD, the antiserum is found to be reactive with mouse IgG1, IgG2a, IgG2b, IgG3, IgA and IgM.

Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis, prior to conjugation. Electrophoresis of the product, followed by diffusion versus anti-rabbit IgG and anti-rabbit whole serum results in single arcs of precipitation.

Titers

1. Direct ELISA: 1:30,000

Microtiter plates are coated with purified mouse IgG at a concentration of 5 µg/ml in 0.05 M carbonate/bicarbonate buffer, pH 9.6 (Carbonate/Bicarbonate buffer capsules are available as Sigma Product No. C-3041). 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°C (Voller, et al.).²

Substrate: *p*-Nitrophenyl Phosphate (pNPP, Sigma Product No. N-2765), 1.0 mg/ml in 10% diethanol-amine buffer, pH 9.8, containing 0.5 mM MgCl₂.

2. Dot Blot: 1:30,000

Diluted conjugate detects 1 ng mouse IgG bound to nitrocellulose.

Substrate: 5-Bromo-4-chloro-3-indolyl Phosphate/ Nitroblue Tetrazolium (BCIP/NBT, SIGMA FAST[™] Tablets, B-5655).

3. Immunohistology: 1:100

Determined by an indirect assay using formalin-fixed, paraffin-embedded sections of human tonsil and Mouse Monoclonal Anti-Human IgM (Sigma Product No. I-6385) as the primary antibody.

Substrate: Fast Red TR/AS-MX Naphthol Phosphate³ (Sigma FAST[™] Tablets F-4523 or F-4648).

4. Western Blotting: 1:30,000

Mouse IgG was detected directly using 10 µg protein. Reducing conditions on an SDS-PAGE gradient (4-20%) gel were used. The protein was transferred to nitrocellulose, blocked with 5% BSA in 0.05 M Tris and then incubated with the conjugate.

Substrate: 5-Bromo-4-chloro-3-indolyl Phosphate/ Nitroblue Tetrazolium (BCIP/NBT, SIGMA FAST[™] Tablets, B-5655).

Working Dilutions

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

References

1. Avrameas, V., Immunochemistry, **6**, 43, (1969).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).
3. Pluzek, K. and Ramlau, R., Alkaline Phosphatase Labeled Reagents, In: CRC Handbook of Immunoblotting of Proteins, Vol. 1: Technical Descriptions, Bjerrum O. and Heegaard, N., (Eds.), CRC Press Inc., Boca Raton, FL, p. 177, 1988.

Storage

Store at 2-8°C. **Do Not Freeze.**

*Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.