3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-Mouse IgG (whole molecule) F(ab′)$_2$ fragment–Alkaline Phosphatase
produced in sheep, affinity isolated antibody

Catalog Number **A3563**

Product Description

Anti-Mouse IgG (whole molecule) F(ab′)$_2$ fragment is produced in sheep using purified mouse IgG as the immunogen. The F(ab')$_2$ fragment of the antibody is obtained from pepsin digested antiserum by immunospecific methods of purification. Affinity isolation removes essentially all sheep serum proteins, including immunoglobulins which do not specifically bind to mouse IgG. Sheep anti-mouse IgG is conjugated to alkaline phosphatase by protein cross-linking with glutaraldehyde.[1]

Identity and purity of the antibody is established by immunoelectrophoresis, prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion against anti-sheep IgG and anti-sheep whole serum results in single arcs of precipitation. The antibody preparation is found to consist only of the F(ab')$_2$ fragment of sheep IgG as determined by SDS-PAGE. No contamination with sheep IgG whole molecule is observed.

Reagents

Supplied as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1 mM $MgCl_2$, 50% glycerol, and 15 mM sodium azide as a preservative.

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/Stability

Store at 2–8 °C.

Product Profile

Dot Blot: recommended working dilution 1:30,000
Diluted conjugate detects 2.1 ng of mouse IgG bound to nitrocellulose.

Substrate: 5-Bromo-4-chloro-3-indolyl phosphate/Nitro Blue Tetrazolium, SIGMA*FAST*™ BCIP/NBT Tablets, Catalog Number B5655.

Direct ELISA: recommended working dilution 1:30,000
Multiwell 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, Catalog Number C3041.

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.[2]

Substrate: 4-Nitrophenyl phosphate (pNPP) disodium salt, Catalog Number N2765, 1.0 mg/ml in 10% diethanolamine buffer, pH 9.8, containing 0.5 mM $MgCl_2$.

Immunohistochemistry: recommended working antibody dilution 1:50.
Determined by an indirect assay using formalin-fixed, paraffin-embedded sections of human tonsil.
Monoclonal Anti-Human IgG, Catalog Number I6760, as the primary antibody.

Substrate: Fast Red TR/Napthol AS-MX, SIGMA*FAST* Tablets, Catalog Numbers F4523 or F4648.[3]

Immunobloting: recommended working dilution 1:30,000. Mouse IgG was detected directly using 10 μg of protein. Reducing conditions on an SDS-PAGE gradient (4–20%) gel were used. The protein was transferred to nitrocellulose, blocked with 0.5% BSA in 0.05 M Tris, and then incubated with the conjugate.

Substrate: 5-Bromo-4-chloro-3-indolyl phosphate/Nitro Blue Tetrazolium, SIGMA*FAST* BCIP/NBT Tablets, Catalog Number B5655.

Note: 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, S., *Immunochemistry*, **6**, 43, (1969).
2. Voller, A., et al., *Bull. World Health Organ*., **53**, 55 (1976).
3. Pluzek, K.J., and Ramlau R., Alkaline Phosphatase Labeled Reagents, in *CRC Handbook of Immunoblotting of Proteins*, **Vol.1**, Bjerrum, O. J., and Heegaard, N. H. H., (Eds.) p. 177 (CRC Press, Inc., Boca Raton, FL, 1988).

TD,KAA,PHC 05/09-1