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-Sheep IgG (whole molecule)–Alkaline Phosphatase
produced in donkey, affinity isolated antibody

Catalog Number **A5187**

Product Description

Anti-Sheep IgG (whole molecule) is produced in donkey using purified sheep IgG as the immunogen. Affinity isolated antibody is obtained from anti-sheep IgG antiserum by immunospecific purification which removes essentially all donkey serum proteins, including immunoglobulins, which do not specifically bind to sheep IgG. Anti-Sheep IgG is conjugated to alkaline phosphatase by protein cross-linking with 0.2% glutaraldehyde.

Specificity of the antiserum is determined by immunoelectrophoresis (IEP), prior to conjugation. By IEP, the antiserum reacts specifically with normal sheep serum and sheep IgG.

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

Reagent

Provided 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.

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

Direct ELISA: Minimum 1:30,000
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 °C [2]
Microtiter plates are coated with purified sheep 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 Cat. No. C3041.

Substrate: *p*-Nitrophenyl Phosphate (pNPP), Cat. No. N2765, 1.0 mg/mL in 10% diethanolamine buffer, pH 9.8, containing 0.5 mM $MgCl_2$.

Dot Blot: Minimum 1:30,000
Diluted conjugate detects 1 ng sheep IgG bound to nitrocellulose.

Substrate: 5-Bromo-4-chloro-3-indolyl Phosphate/Nitroblue Tetrazolium (BCIP/NBT), SIGMA*FAST*™ Tablets, Cat. No. B5655.

Western Blotting: Minimum 1:30,000
Sheep IgG was detected directly using 10 µg protein per lane. Reducing conditions on an SDS-PAGE gradient (4-20%) gel was 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, Cat. No. 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, V., *Immunochemistry*, **6**, 43, (1969).
2. Voller, A., et al., *Bull. World Health Organ.*, **53**, 55 (1976).
3. Pluzek, K., and Ramlau, R., Alkaline Phosphatase Labeled Reagents, In: CRC Handbook of Immunoblotting of Proteins, Bjerrum O., and Heegaard, N., (Eds.), CRC Press Inc., Boca Raton, FL, **1**, p. 177, 1988.

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