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Product Information

Anti-Pig IgG (whole molecule)–Alkaline Phosphatase produced in rabbit, affinity isolated antibody

Catalog Number **A1192**

Product Description

Antiserum is produced in rabbit using purified pig IgG as the immunogen. Affinity isolated antibody is obtained from anti-pig IgG antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to pig IgG. Anti-Pig IgG is conjugated to alkaline phosphatase by protein cross linking with 0.2% glutaraldehyde.[1]

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

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.

Reagent

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

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.

Product Profile

Dot Blot: minimum dilution1:30,000
Diluted conjugate detects up to 20 ng pig IgG bound to nitrocellulose.

Substrate: 5-Bromo-4-chloro-3-indolylphosphate/ nitroblue tetrazolium (BCIP/NBT), SIGMA*FAST*™ Tablets, Catalog Number B5655.

Direct ELISA: minimum titer 1:35,000
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 pig 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.

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

Western Blotting: minimum dilution 1:30,000
Pig IgG was detected directly using 10 µg protein per lane. 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, transferred and then incubated with the conjugate.

Substrate: BCIP/NBT, SIGMA*FAST*™ 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, V., *Immunochemistry*, **6**, 43, (1969).
2. Voller, A., et al., *Bulletin WHO*, **53**, 55 (1976).
3. Pluzek, K.J. and R. Ramlau, Alkaline Phosphatase Labeled Reagents, in CRC Handbook of Immunoblotting of Proteins, O.J. Bjerrum and N.H.H. Heegaard, Eds., CRC Press Inc., Boca Raton, FL, **1**, p. 177, 1988.

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