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ProductInformation

ANTI-MOUSE IgG (WHOLE MOLECULE)
BIOTIN CONJUGATE
F(ab')$_2$ Fragment of
Affinity Isolated Antigen Specific Antibody

Product No. **B 4765**

Product Description

Antiserum is developed 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, that do not specifically bind to mouse IgG. Sheep anti-mouse IgG is conjugated to Sigma N-Hydroxysuccinimidobiotin (Product No. H 1759) by a modification of the method of Bayer, et al.[1]

Specificity

Specificity for mouse IgG is determined by immuno-electrophoresis (prior to conjugation) versus mouse IgG and normal mouse serum. The antibody preparation is reactive purified mouse IgA, IgG1, IgG2a, IgG2b, IgG3, and IgM myeloma proteins as determined by Ouchterlony Double Diffusion (ODD) prior to conjugation.

Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis (IEP), prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion versus 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-Polyacrylamide Gel Electrophoresis (PAGE). No contamination with sheep IgG whole molecule is observed.

Antibody Content

The product is provided with a specific antibody content of 0. 1 to 2.0 mg/ml (prior to the addition of BSA).

Working Dilution: Minimum 1:20,000
Working dilution is defined as the dilution of conjugate that gives a change in absorbance of 1.0 at 492 nm after 30 minutes of substrate conversion at 25 °C (Voller, et al. and Guedson et al.).[2,3] Microtiter plates are coated purified mouse IgG at a concentration of 200 ng/ml in 0.05 M carbonate/bicarbonate buffer pH 9.6 (carbonate/bicarbonate buffer capsules are available as Product No. C 3041). Following incubation with the biotinylated antibody a solution of Avidin-Horseradish Peroxidase (Product No. A 3151, diluted in 0.01 M phosphate buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.5% BSA) is added.

Substrate: *o*-Phenylenediamine Dihydrochloride (OPD, Sigma Product No. P 8287), 0.4 mg/ml in 0.05 M phosphate-citrate buffer, pH 5.0 containg 0.03% sodium perborate (phosphate-citrate buffer capsules with sodium perborate are available as Product No. P 4922).
**Add immediately before use.

Reagents

The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

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.

Storage

For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

1. Bayer, E.A., et al., Methods in Enzymology, **62**, 308 (1979).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).
3. Guedson, J.L., et al., J. Histochem. and Cytochem., **27**, 1131 (1979).

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