

Product No. B 7015 Lot 047H8808

Anti-Pig IgG (whole molecule) Biotin Conjugate Antibody developed in Rabbit Affinity Isolated Antigen Specific Antibody

Antiserum is developed in rabbit using purified pig IgG as the immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-pig IgG antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immuno-globulins, that do not specifically bind to pig IgG. Rabbit Anti-Pig IgG is conjugated to Sigma N-Hydro-xysuccinimidobiotin (Sigma Product No. H 1759) by a modification of the method of Bayer, et al.<sup>1</sup> The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15mM sodium azide (see MSDS)\* as a preservative.

## Specificity

Specificity of the anti-pig IgG antibodies for pig IgG is determined by immunoelectrophoresis (IEP) prior to conjugation using normal pig serum and pig IgG.

## **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-rabbit IgG and anti-rabbit whole serum results in single arcs of precipitation.

# **Antibody Content**

The product is provided with a specific antibody content of 0.34 mg/ml (prior to the addition of BSA).

## Working Dilution: 1:48,000

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  $25^{\circ}$ C (Voller, et al. and Guedson et al.)<sup>2,3</sup>. Microtiter plates are coated

purified pig 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 Sigma Product No. C 3041). Following incubation with the biotinylated antibody, a solution of Avidin-Horseradish Peroxidase (Sigma 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 containing 0.03% sodium perborate (Phosphate-Citrate Buffer Capsules with Sodium Perborate are available as Sigma Product No. P 4922).

#### Storage

For continuous use, store at 2-8°C. 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., et al., Methods in Enzymology, **62**, 308 (1979).
- 2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).
- Guedson, J., et al., J. Histochem. and Cytochem., 27, 1131 (1979).

\*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.

Sigma warrants that its products conform to the information contained in this and other Sigma publications. Purchaser must determine the suitability of the products for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale. Issued 06/97.