

Product No. A-6792

Lot 026H8864

**Anti-Dog IgG (whole molecule)
Peroxidase Conjugate**

Antibody developed in Rabbit
Affinity Isolated Antigen Specific Antibody

Antiserum is developed in rabbit using purified dog IgG as the immunogen. Antibody is isolated from rabbit anti-dog IgG antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins that do not specifically bind to dog IgG. Rabbit anti-dog IgG is conjugated to Sigma Horseradish Peroxidase, Type VI (Sigma Product No. P-8375) by a modification of the periodate method of Wilson and Nakane.¹ The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.01% thimerosal as a preservative.

Specificity

Specificity of the anti-dog IgG antiserum is determined by immunoelectrophoresis, prior to conjugation, versus normal dog serum and dog 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.

Titer: 1:12,000 (Direct ELISA)

We are now reporting lot specific information as a titer by direct ELISA rather than as a working dilution. Titer is defined as the dilution of conjugate sufficient to give a change in absorbance of 1.0 at 450 nm after 30 minutes of substrate conversion at 25°C (Voller, et al.²). Microtiter plates are coated with purified dog 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 Sigma Product No. C-3041).

Please see below for working dilution.

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).

Working Dilution

Working dilution should be determined by titration assay. Due to product improvement and changes in the assay procedure, we now list a lot specific titer by direct ELISA for this product. Due to differences in assay systems, this titer may not reflect the user's actual working dilution.

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. Wilson, M., and Nakane, P., In: Immunofluorescence and Related Staining Techniques (Elsiever/North Holland BioMedical Press, Amsterdam), p215 (1978).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).

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