**Product No. A-8400**
**Lot 036H8825**

# Anti-Human Polyvalent Immunoglobulins (IgA, IgG, IgM) Peroxidase Conjugate

Antibodies developed in Goat
IgG Fraction of Antisera

Individual antisera to human IgA, IgG and IgM are developed in goats using purified human IgA, IgG and IgM as the immunogens. Whole antisera are fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of each antiserum. This fraction is essentially free of other goat serum proteins. Each fraction is then 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 for each immunoglobulin is determined by Ouchterlony double diffusion (ODD) and immunoelectrophoresis (IEP) versus purified human IgA, IgG, IgM, Bence Jones kappa and Bence Jones lambda myeloma proteins prior to conjugation.

## Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis, prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion versus anti-goat IgG and anti-goat whole serum results in single arcs of precipitation in the gamma region.

**Titer:** 1:5,000 (Direct ELISA, each Ig)

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 individually coated with purified human IgA, IgG or IgM 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., Immunofluorescence and Related Staining Techniques (Elsevier-North Holland BioMedical Press, Amsterdam), p. 215 (1978).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).