

**Product No. A-0668**

**Lot 044H4803**

**Anti-Bovine IgG (whole molecule)  
Alkaline Phosphatase Conjugate**  
Antibody Developed in Rabbit  
IgG Fraction of Antiserum

Antiserum is developed in rabbit using IgG isolated from pooled normal bovine serum as the immunogen. Whole antiserum is fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of antiserum. This fraction is essentially free of other rabbit serum proteins. Rabbit anti-bovine IgG is conjugated to Sigma Alkaline Phosphatase using 0.2% glutaraldehyde. The conjugate is provided as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, and 1 mM MgCl<sub>2</sub>, with 0.1% sodium azide (see MSDS)\* as preservative.

#### **Specificity**

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

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

**Titer:** 1:10,000 (Direct ELISA)

Titer is defined as the dilution of conjugate sufficient to give a change in absorbance of 1.0 at 400 nm after 30 minutes of substrate conversion at 25°C (Voller, et al.<sup>1</sup>). Microtiter plates are coated with purified bovine IgG at a concentration of 20 µg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide.

**Substrate:** *p*-Nitrophenyl phosphate (pNPP, Sigma Product No. N-2765), 1.0 mg/ml in 10% diethanolamine buffer, pH 9.8, containing 0.01% MgCl<sub>2</sub> and 0.02% NaN<sub>3</sub>.

#### **Dot Blot**

1. A 1:20,000 dilution in a direct assay using 20 ng bovine IgG/dot.
2. A 1:100,000 dilution in an indirect assay using 20 ng rabbit IgG/dot and bovine anti-rabbit IgG as the first antibody.

**Immunohistology:** 1:50

Determined by an indirect assay using formalin-fixed, paraffin-embedded rabbit spleen and bovine anti-rabbit IgG as the first antibody.

#### **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**

Store at 0-5°C. **Do Not Freeze.**

#### **Reference**

1. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).

\*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.

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