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# **ProductInformation**

## Protein A - 20 nm Colloidal Gold Labeled

Product Number P 6855 Storage Temperature 2-8 °C

# **Product Description**

Protein A - 20 nm Colloidal Gold Labeled is prepared with extracellular Protein A from *Staphyloccus aureus* (Product Number P 6031) that is adsorbed to colloidal gold. It is suitable for detection of immunoglobulins.

Colloidal gold is an electron-dense, non-fading marker useful as a probe in electron microscopy (TEM and SEM), light microscopy, and blotting.<sup>1</sup> It requires no additional processing for detection, but in some applications the signal can be enhanced by reaction with silver.<sup>2,3</sup> It can be complexed with biomolecules by strong, non-covalent interactions.<sup>4</sup>

Generally, particles < 15 nm are most useful in transmission electron microscopy (TEM) and for applications where access to the probe may be hindered by larger particles.<sup>5</sup> Particles >15 nm are more suitable for scanning electron microscopy (SEM), light microscopy, and blotting.

## Reagent

Protein A - 20 nm Colloidal Gold Labeled is supplied as a clear, red, collodial suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 20% glycerol, 1% bovine serum albumin, and 15 mM sodium azide.

Particle size: 20 nm (Mean: 17 to 23 nm).

Monodispersed with ≥ 60 % singlets

Gold conjugates are processed, after adsorption with proteins, to remove free protein and large aggregates. The concentration is expressed as absorbance at the absorption maximum  $(A_{520})$ .<sup>2, 6</sup>

## **Precautions and Disclaimer**

Due to the sodium azide content a material safety 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.

## **Preparation Instructions**

The product should be diluted for most applications. It is recommended that diluent buffer contain 0.15 M saline buffered at pH 6 to 8, with 0.5% albumin (Product No. A 7638) and 0.05% TWEEN® 20 to minimize background (additional buffer supplement may be required for certain applications, see "dot blot" procedure). It is also recommended that, prior to application, the diluted conjugate be allowed to equilibrate at least 20 minutes in the diluent buffer, which has a lower glycerol content than the product buffer. The optimal concentration of the conjugate must be determined empirically, dependent on specific usage, and it generally ranges from final  $A_{520} = 1.0$  to 0.05 (2.5 to 50-fold dilution) with incubation times ranging from 0.5-12 hours.

## Storage/Stability

Store at 2-8  $^{\circ}$ C. Freezing will cause aggregation of the colloid.

## **Procedure**

Binding is evaluated by a modified "dot blot" assay. Serial dilutions are prepared from a 1 mg/ml positive control (human IgG) protein solution. One  $\mu$ I of each dilution is adsorbed onto a nitrocellulose membrane and allowed to dry. The gold conjugate is diluted to A<sub>520</sub> = 0.25 (approximately a 10-fold dilution) with 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.0, 5 mg/ml albumin, and 0.05% TWEEN 20. The spotted membranes are incubated with the gold conjugate for 1 hour at 25 °C. The detection limit is the minimum amount of protein that can be detected as a pink-red spot on the membrane.

## **Product Profile**

Protein A - 20 nm Colloidal Gold Labeled will detect 30 ng or less of human IgG by dot blot.

#### References

- Beesley, J.E., Proc. Royal Micr. Soc., 20, 187 (1985).
- 2. Brada, D., and Roth, J., Anal. Biochem., **142**, 79 (1984).
- 3. Danscher, G., and Norgaard, J.D., Histochem. Cytochem., **31**, 1394 (1983).
- 4. Geoghegan, W.D., et al., Immunol. Comm., 7, 1 (1978)
- 5. Slot, J.W., and Geuze, H.J., J. Cell. Biol., **90**, 533 (1981).

- 6. Tolson, N.D., et al., J. Microsc., **123**, 215 (1981).
- 7. Ackerman, G.A., et al., J. Histochem. Cytochem., **31**, 433 (1983).
- 8. Bendayan, M., J. Electron Microscopy Technique, 1, 243 (1984).
- 9. Bendayan, M., J. Histochem. Cytochem., **30**, 81 (1981).

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