BBINTERNATIONAL
Data Sheet and Technical Information

EM GRADE GOLD CONJUGATE EM GOAT ANTI-HUMAN IgG: 20nm GOLD BATCH 3517

Every batch of EM grade gold conjugates is thoroughly tested with an exhaustive series of quality control procedures:
In order to ensure compatibility with other sizes in the BBI conjugate range for double or triple labelling of antigens, the mean diameter and coefficient of variation for every batch is accurately determined by high resolution electron microscopy. The coefficient of variation must not exceed 12% of the mean diameter for 5nm, or 8% of the mean diameter for 10, 15 and 20nm particles, or 15% of the mean diameter for 30 and 40nm particles.

For quantitative analysis and maximum sensitivity it is important to obtain labelling free from aggregates formed in the conjugate. With Protein A, Protein G and Protein AG conjugates, clustering in the final labelling is generally much lower than with IgG conjugates. This is not related to the quality of the conjugate itself but to the natural one-to-one affinity of Protein A with the primary antibody, compared with the amplifying effect of IgG conjugates on the primary antibody. True clustering in BBI gold conjugates, however, is virtually absent when examined by layering onto lysine in the electron microscope. All BBI gold conjugates must exhibit very low levels of doublets and must be totally free from any clusters greater than triplets. Thus for EM grade conjugates these critical controls allow only the best fractions to be retained from each batch during manufacture.

If particle sizing and clustering are found to be acceptable, each batch of conjugate is further tested for sensitivity by incubations with target protein dot blots to determine the minimal detectable amount of protein. In this procedure the specific protein is serially diluted and dot blotted onto nitrocellulose membrane, then incubated with the gold conjugate. To make the gold conjugate visible, the strips are silver enhanced. Detection sensitivity of at least 10pg is required for IgG conjugates using silver enhancement. Protein A, Protein G and Protein AG are set at 100pg after silver enhancement. For lectins a sensitivity of 100pg of sugar is required using silver enhancement.
Specification and quality control data as shown below for: **EM.GAH20**

Optical Density: 4.0

BBI Conjugates are presented in a highly concentrated form and are thus extremely economical to use. Conjugates with 5 and 10nm particles are manufactured to an optical density of 3.0 at 520nm. For 15 and 20nm the optical density is 4.0, and for 30 and 40nm the optical density is 5.0. Dilution of the conjugates for EM incubations is generally between 1/10 and 1/100 in the appropriate buffer.

Protein concentration:
Final concentration of protein in the gold conjugate: 17.2µg/ml

Particle Size Distribution:
The conjugate's particle diameter is measured at high magnification in the electron microscope.

No. of particles measured:	100
Mean diameter:	20.1nm
Coefficient of variation:	2.4 %

Minimal detectable protein:
Aliquots of 1µl of Human IgG were blotted onto nitrocellulose strips and incubated for 2 hours in 1/40 dilution of the conjugate. The strips were then further developed in silver enhancing solution to produce black deposits at sites of gold conjugate.

Minimum detectable protein after silver enhancement: 400pg

Particle Clustering:
The conjugate was layered onto poly-L-lysine on Formvar coated grids and the particle clustering determined at high magnification in the electron microscope.

% of single particles	98 %
% of particles larger than triplets	0 %

Buffer:
The conjugate is provided in Tris buffered saline, pH 8.2, containing 20% glycerol, 1% bovine serum albumin and with 0.1% sodium azide as preservative.

Stability:
Stability of the conjugates is good for at least one year at 4°C. The conjugates are also remarkably stable at ambient temperatures. Repeated freezing and thawing is not recommended. Conjugates with glycerol may be aliquoted and frozen for long-term storage. The glycerol may be subsequently removed by dialysis if necessary.

Quality control approved by.....