TECHNICAL DATA SHEET

SPI Supplies
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SPI-Mark™ Gold Conjugates for Electron Microscopy

Gold Conjugates for Electron Microscopy

While most immuno gold work, with electron microscopy has been done in conjunction with TEM, there have been published some very exciting results based on the use of SEM as well. For example, there is great interest in using colloidal gold tagged antibodies for identifying surface antigens on the surfaces of cultured cells. While the precise details of the protocol one would end up using is very much dependent on the specifics of the samples, we believe that one of the best publications on this topic is the following:

Coller, Barry S., Kutok, J. L., Scudder, L. E., Galanakis, D. K., West, S. M ., Rudomen, G. S., Springer, K. T., "Studies of Activated GPIIb/IIIa Receptors on the Luminal Surface of Adherent Platelets: Paradoxical Loss of Luminal Receptors When Platelets Adhere to High Density Fibrinogen." J. Clin. Invest. Vol. **92**, pp.2796-2806, 1993.

Concentration issues:

Everyone has at least some interest in the concentration of the gold colloid and conjugate in the suspension being purchased. Most of this information has been gathered on the unconjugated gold probes but it would be applicable to the conjugates as well. How all this translates down into what it means in terms of the number of protein molecules per gold particle, consider these examples for Protein A:

1. The 1 nm particles will have 1 or 2 gold particles per protein molecule, and
2. The 5 nm particles will have ~ 4 Protein A molecules per one gold particle.
3. The 20 nm particles will have 48 Protein A molecules per one gold particle.

The number of gold particles per ml is another question that is sometimes asked. We don't have measurements on all gold sizes, but an example is given for 20 nm gold conjugates:

For an optical density (OD) of 4: 2.8×10^{-12} particles per ml.

Note: Because of some technical difficulties, we are temporarily unable to offer 1 nm gold products, either conjugated or unconjugated, but the example for 1 nm has been left in the table for illustrative purposes.

Measurements have also been made on antibody conjugates.

1nm = 1 to 3 Gold Particles per IgG antibody
5nm = 3 antibodies
10nm = 12 antibodies
15nm = 27 antibodies
20nm = 48 antibodies
30nm = 100 antibodies
40nm = 160 antibodies

The larger the particle the greater the surface area therefore the greater the number of antibodies that can bind. Ultimately, it is believed that there is some binding of the Fab region and that some binding sites are not going to be available. Some antibodies will give better sensitivity than others because the amino acids in their Fab fragments don't bind and therefore the Fabs are free. If sensitivity is poor, it may be worth examining antibodies from other host species, as each will have different characteristics.

Protein A is a lot smaller than IgG, therefore there would be less gold particles of 1nm gold attached to Protein A, and more Protein A molecules attached to the larger particles sizes. A 20nm gold particle would have something like 150 Protein A molecules compared to the 48 IgG's.

Quite a bit of time, effort, and energy is spent characterizing such issues for the SPI-Mark Gold Probes, so if you have additional questions, let us know, but with the caveat that what we tell you about the SPI-Mark brand of colloidal gold probe suspensions would not be directly applicable to other brands.

Color considerations:

Questions often times come up about what should be the "correct color". In general, the SPI-Mark Colloidal Gold products, like all other colloidal gold products (to our knowledge) are that deep "Cabernet Sauvignon" red or almost purple color *except* for the SPI-Mark 2 nm gold products. Both are pale straw in color, and look brown when concentrated x10. If one ends up with a water white clear solution and they don't now if there is any gold present or not, then the conclusive "test" is to blot 1µl of each onto a nitrocellulose membrane filter and silver enhancing. If the clear solutions don't enhance, then there are no gold particles present.

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