TECHNICAL DATA SHEET

SPI Supplies
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SPI-Mark™ Unconjugated Colloidal Gold Probes

Gold Colloids Unconjugated

SPI-Mark™ unconjugated gold colloidal suspensions are of outstanding EM quality, exhibit virtually no clumping and exhibit very tight size distribution ranges, so tight in fact, that triple tagging experiments are no problem at all. Numerous protocols have now been published, and many are application specific, but for the convenience of SPI-Mark customers, we provide a generalized protocol, but it is given more for the purpose of showing generally what is involved, rather than to serve as a specific protocol for a specific experiment.

Sample protocol for conjugation:

1. Clean all glassware (most important!).
2. Prepare aqueous protein solution at 0.1mg/ml.
3. Adjust SPI-Mark colloidal gold suspension pH > isoelectric point of protein.
4. Add enough protein (determined by titration) to stabilize the gold colloid. SPI-Mark Silver Enhancement Kit is required for 2nm and sometimes for 5 nm.
5. Add BSA to a concentration of 0.1%.
6. Centrifuge to concentrate and remove excess protein.
7. Centrifuge over a glycerol gradient to remove clusters.
8. Pool fractions and dilute to 30mg/ml protein in a suitable buffer for storage.

The starting material for the SPI-Mark gold conjugates is the same as what is offered here as unconjugated gold colloidal suspensions. In other words, these colloids are produced with a very tight size distribution, the actual size being measured by TEM and the data is provided on the data sheet sent with each product. The product is supplied in sterile containers ready for conjugation or other applications, such as perfusion.

Shelf-life stability of the colloid:

The product is guaranteed stable for 12 months if stored unopened at 4° C. *Do not freeze*. Note that we do not offer unconjugated gold in sizes less than 2 nm because the stability of such suspensions is unacceptable in terms of offering such an unstable product commercially. Once made, the unconjugated colloid has to be used within 24 hours. That is why gold of 1 nm is offered only as a stable conjugate, never as the unconjugated colloid.

Other sometimes useful information:

Colloidal particles in this size range, including gold, are inherently unstable. However, using proprietary technology, SPI-Mark colloidal gold unconjugated particles have no protein whatsoever and are free of surfactants, both of which can interfere with experimental results. We are often times asked about "what else" is present besides the gold colloid and water. There will be trace amounts of sodium citrate, tannic acid & potassium carbonate present and without them, the suspension would become unstable and the gold would precipitate out.

However, the smallest colloidal particle that we can make and still be stable, using this technology is 2.6 nm. So all SPI-Mark unconjugated gold products, of 2.6 nm or larger are indeed free of surfactants and protein, as indicated above. But should we be asked to custom make gold colloid particles, unconjugated, smaller than this size, then there would be a protein coating such as BSA.

Amount of gold present:

Each liter of colloidal suspension contains approximately 0.1g of gold (colloidal) particles. The final product is sold with an optical density as measured at 520 nm of 1.1 ±0.1 for colloid sizes of 15, 20, 30, 40 and 50 nm. The optical density for smaller sizes, that is, 2, 5 and 10 nm, is 0.85.

There is one other important point to note:

The basic for the technology that permits the manufacture of stable suspensions (e.g. one in which the particles don't clump) without a stabilization coating is the overall net charge on the colloidal particle surfaces is negative, and this provides the mechanism by which particles repel one another and the suspension remains stable. However, excessive washing of the colloid can remove or destroy this charge and the stable suspension will collapse. It should be further noted that the 2.6 nm size is "at the edge" that is, even the slightest bit of washing of the colloid will cause the collapse of the suspension immediately. However, dilution of the suspension is possible with ultra pure water. This is possible because of the extremely low levels of the reducing salts remaining in the product.

Stability information:

The SPI-Mark gold suspensions are very stable because all of the gold, in the manufacturing process, has been reduced to the colloidal metal form with absolutely no gold left in solution. In addition, there are only trace amounts of the reducing salt used in the conversion. Higher amounts of the reducing salts are one of the main reasons for product instability that have been encountered with colloidal gold suspensions from other sources. Unconjugated colloids are unstable in salt solutions. They will not immediately collapse but at 150mm there will be a gradual moving together of the particles as the ionic distribution around the particles is altered. There is nothing keeping the particles from touching.

One can coat the particles and then add salt. If you want to avoid protein, then a polymer will work, but it must be remembered that unless the polymer is adsorbed onto the surface it will merely be keeping the particles apart by intervention and will only delay the collapse by salt solution. Polymers which may be suitable for adsorbing onto the surface will include any which

have a hydrophobic or sulphur content. Many polymers have been used historically with mixed results, eg PVP, PVA, PEG. A polymer which incorporates a thiol group could prove the best because of the interaction between sulphur and gold.

A description of the binding forces between gold particles and molecules is given in the IVD article "The Place of Gold in Rapid Tests", J Chandler, T Gurmin and N Robinson, **IVD Technology**, March/April, 2000, p37-49.

Selection of the right colloid gold particle size:

The entire product range of the SPI-Mark unconjugated colloidal gold particles is given below. The selection of the right particle size depends on the your specific application and the way you would be anticipating using the product.

- **Light Microscopy**
  For light microscopy, in conjunction with silver enhancing, the smaller sizes are recommended (2nm and 5nm). As a general rule of thumb, smaller particles give greater specificity. Also, smaller particle suspensions are inherently more stable and have somewhat longer shelf lives.

Colloidal Gold Size	Particle Size Distribution (% CV)	Particles per ml
2	-	15×10^{13}
5	< 15%	5×10^{13}
10	< 10%	5.7×10^{12}
15	< 10%	1.4×10^{12}
20	< 15%	7.0×10^{11}
30	< 20%	2.0×10^{11}
40	< 20%	9.0×10^{10}
50	< 20%	4.5×10^{10}
60	< 20%	2.6×10^{10}
80	< 20%	1.1×10^{10}
100	< 20%	5.6×10^{9}
150	< 20%	1.7×10^{9}
200	< 20%	7.0×10^{8}
250	< 20%	3.6×10^{8}

- **Electron Microscopy (TEM and SEM)**
  For transmission electron microscopy (TEM) any particle size may be used, the smaller particles giving the highest labeling specificity (efficiency). The magnification required for the TEM study determines the particle size to be used. Smaller particles may be silver enhanced using the SPI-Mark Silver Enhancement Kit for viewing at lower magnifications.

  For scanning electron microscopy (SEM), particles of 20nm, 30nm or 40nm may be used together with backscattered electron (BSE) imaging. However, smaller particles will give a higher labeling intensity and may be silver enhanced.

- **Blotting procedures**
  For blotting applications, the larger particles give the most visible stain without silver enhancing. Larger particles may produce more steric hindrance to the labeling, however, resulting in a lower specificity of the experiment. The compromise is usually between 20-40nm for most blotting applications. If silver enhancement is contemplated, we recommend, for the best results, the 2nm or 5nm gold particles.

Restriction of use statement:

The SPI-Mark Unconjugated Colloidal Gold is for *in vitro* research only and it is most definitely not for *in vivo* research. SPI Supplies cannot take any responsibility for the use of this product family in human or animal research and would not recommend or condone it.

For your information the colloid will contain trace elements of sodium citrate, tannic acid & potassium carbonate. The toxic effects of these trace chemicals and the effect of the colloid in this way on rats has not, to my knowledge, been tested.

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