Physisorption of Antibodies using BioReady Bare Nanoparticles

Introduction

For more than 20 years, lateral flow immunoassay diagnostic tests have provided a simple, self-contained, portable device format that is easy to use, fast and inexpensive. These devices can be stored at ambient temperature, have a long shelf life, and provide diagnostic results within minutes without sample processing or additional equipment. Billions of tests are produced each year to detect a variety of analytes in point-of-care and field-based diagnostics.

Figure 1.The commercial pregnancy test uses gold nanoparticles for detection. The faint red line indicates a positive result. The dark red line is the control.

The most common lateral flow assay configuration uses colloidal gold nanoparticles to signal the detection of an analyte, such as the commercially available pregnancy test. The lateral flow device has a reading window for viewing the results at the test and control lines (Figure 1). To yield a signal, the nanoparticle must be conjugated to a molecular recognition element, which is typically an antibody. The surface of gold nanoparticle probes naturally adhere proteins. Therefore, adding antibodies to a solution of gold nanoparticles will often result in the physical adsorption to the nanoparticle surface (i.e., physisorption). To maximize the sensitivity and specificity of the lateral flow assay, the assembly of antibodies on the gold nanoparticles needs to be optimized. Factors that require simultaneous optimization include the buffer pH, salt concentration, antibody/particle ratio and blocking proteins. One of the challenges for assay development is that any lot-to-lot change in the particle size, shape or surface may require re-optimization, resulting in a loss of time and profit. A new product family of gold nanoparticles has been optimized to maximize performance and reliability for lateral flow assays that use physisorption to prepare detector particles.

Nanoparticle Surface

The self-assembly of antibodies and other proteins on the surface of a gold nanoparticle is difficult to control. Small changes in the pH or salt conditions can alter the charge, hydrophobicity, or structure of the antibody, which can affect the antibody density and orientation on the particle surface. The gold particle surface has exposed crystals with various orientations. Each orientation can have a different affinity for different portions of the antibody. For all physisorption processes, the starting particle should be bare, which is defined as not having any molecular ligands bound to the particle surface. Technically, it is not possible to have a stable bare nanoparticle because the charge of the colloidal double layer keeps the particles from aggregating. Therefore, to produce particles that are as bare as possible, ultra-high purity nanoparticles are fabricated and suspended in a buffer only with components that weakly associate with the surface. In the presence of an antibody or other protein, the protein will displace the weakly associated molecules and bind to the particle surface.

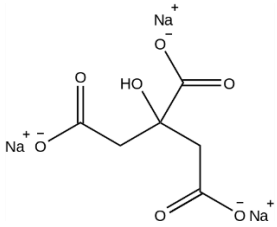

Figure 2. Trisodium citrate is used as a reductant in many gold syntheses and also acts as a stabilizing molecule and buffer component in many gold nanoparticle solutions used for physisorption of antibodies.

The most common buffer for bare nanoparticles is citrate (Figure 2). Sodium citrate is used as a reductant in many gold nanoparticle fabrication methods and provides a balance between stability during particle formation and displaceability when making particle conjugates. Each of the three carboxylic acids weakly bind to the particle surface but are readily displaced in the presence of a protein. nanoComposix offers BioReady™ 40 nm diameter gold nanoparticles suspended in a weak (0.02 mM) sodium citrate buffer. The particles are made in large lots, extensively washed in ultra high purity water to remove residual reactants, and are a drop-in replacement for gold nanoparticles that are currently being used in many commercial assays. Additionally, nanoComposix offers gold nanoparticles in a carbonate buffer (Figure 3). Carbonate is a smaller and less complex molecule and has a lower affinity to the gold nanoparticle surface than citrate. The greater displaceability of the

carbonate molecules typically produces better performing conjugates, and therefore, carbonate-buffered gold particles are preferable for physisorption experiments.

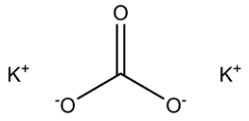

Figure 3. Carbonate molecule has a weak affinity for the particle surface and is the preferred surface for generating high quality physisorbed conjugates.

Optical Properties of Gold Nanoparticles

Gold nanoparticles have unusual and unique optical properties that make them exceptional detector particles for highly sensitive diagnostic tests. When illuminated, gold nanoparticles strongly couple to specific light wavelengths, resulting in high per-particle absorption. The strong light absorbance is from the particle's plasmon resonance, which is a function of the properties of gold and the particle's size and shape. For example, the peak absorptions for the 40 nm and 80 nm diameter particles are ~523 nm and ~546 nm, respectively (Figure 4).

The particle spectra provides information about the particles and how they change with time in different conditions. The maximum value, location and width of the peak are a function of the particle's diameter and the size distribution of the particle population. An elevated baseline at wavelengths >700 nm indicates that the particles have aggregated. It is therefore important to monitor the UV-visible spectra as a function of time or when comparing different lots because changes to the peak height, position, width, and long wavelength baseline indicate that the particles have changed.

For lateral flow applications, gold nanoparticles are supplied in optical density (OD) units, which is a measure of the amount of light that the particles absorb or scatter at a 1 cm path length. This metric is convenient because it is easy to dilute a concentrated nanoparticle suspension to exactly 1 OD. Note that there is batch-to-batch variance in the optical density per unit mass of gold, depending on the particle's exact size, shape and size distribution. Therefore, two different solutions from the same source at 1 OD could have a different particle number per volume and, more importantly, different surface areas per unit volume. Because the antibody/particle ratio is typically determined by surface area, it is important to account for this factor when using a new lot from the same source. The nanoparticles from nanoComposix are measured using both OD and mass concentration (via ICP-MS) for each lot. These data enable any necessary protocol adjustments. The optical specifications for the 40 nm BioReady™ Bare gold nanoparticles are provided in Table 1.

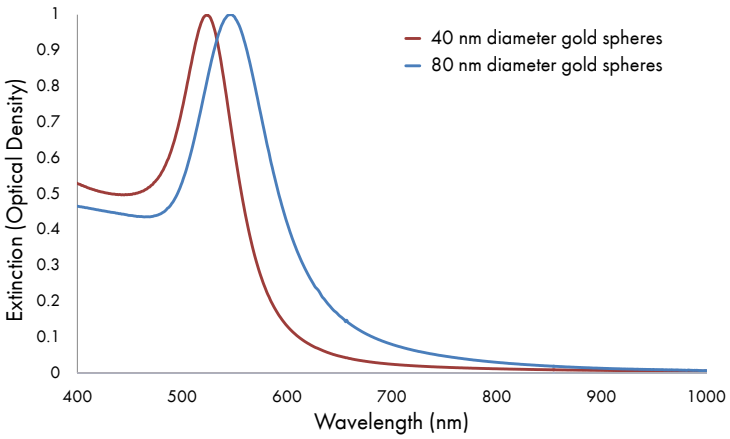

Figure 4. UV-Visible spectra of 1 OD 40 nm and 1 OD 80 nm gold nanoparticles.

Table 1. Optical properties of the 40 nm gold nanoparticles.

Property	Range	Description
Peak Optical Density / mg	21 – 25 OD / mg	A measure of the optical absorption per unit mass. Highly monodisperse particle solutions will have a higher OD/mg and a narrower peak. If the absorbance variance for each particle isn't addressed, then gold solutions provided at the same OD could have different particle concentrations/surface areas, which significantly impacts lot-to-lot variability.
Peak wavelength position	519 – 527 nm	Peak wavelength is a function of size. If the peak particle wavelength is not within this range, the particles might have aggregated.
Optical Density at 700 nm / Optical density at Maximum	< 2.75% of peak OD at 700 nm	There should be only small absorption levels at wavelengths >700 nm. If this baseline is elevated, it is likely that the particles have flocculated or partially aggregated.
Optical Density at 550 nm / Optical density at 600 nm	> 4.3	A measure of the narrowness of the gold nanoparticle peak. This metric ensures that there is a narrow distribution of particle diameters.

Physical Properties of Gold Nanoparticles

In addition to optical properties, other particle characteristics are critical for ensuring lot-to-lot uniformity. A comprehensive certificate of analysis is provided with each BioReady™ Bare gold nanoparticle lot (Figure 5). Transmission electron microscopy (TEM) is used to determine the average and standard deviation of the nanoparticle diameter. The TEM also reveals the shape uniformity of the particles. In many gold nanoparticle synthesis recipes, the particles are faceted and have shape impurities (e.g., triangles, doublets, rods), which can introduce variability into antibody/particle ratios and broaden the spectral peak.

The particle concentration is calculated by TEM diameter, and gold mass concentration is measured by ICP-MS. Based on the TEM-calculated volume, a gold mass per particle can be determined. The ICP-MS concentration is used to calculate particle concentration and surface area. Dynamic light scattering is used to measure the hydrodynamic particle size in solution. A hydrodynamic diameter within 4 nm of the TEM determined diameter verifies that the particles are not aggregated. Lastly, the particle charge (Zeta potential) is measured to ensure that the surface is strongly negatively charged.

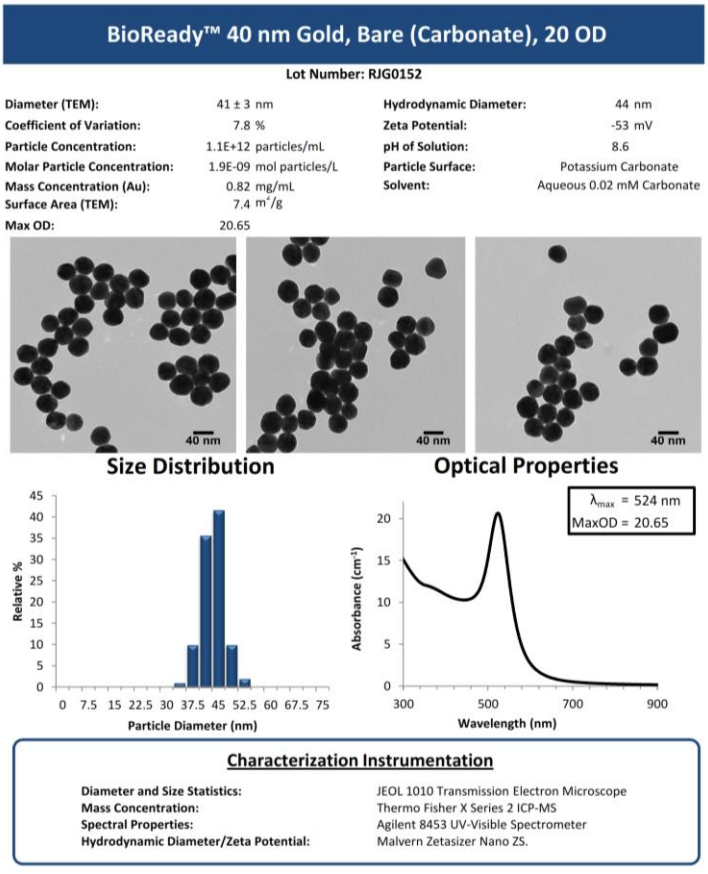

Figure 5. Example certificate of analysis provided with every lot.

To remove residual reactants from the synthesis process, the 40 nm particles are extensively washed using continuous flow filtration with 10 volume washes against a low concentration citrate buffer (0.02 mM citrate) or 7 volume washes into water for the carbonate stabilized particles (<1 pM carbonate). Because the

particles are weakly buffered and carbonic acid will form if the solutions are exposed to air, the pH can change with time. This is the reason that the incoming specification for pH is relatively broad (pH 5.5 – 8.0). However, since the material is essentially unbuffered, the pH is easily adjusted via addition to a conjugation solution or manually adjusted with different buffers.

BioReady™ Bare Products

The BioReady™ bare products are available in 40 nm and 80 nm diameter particles (Table 2). The 40 nm diameter particles are provided at a concentration of 20 OD, which is approximately 1 mg/mL of gold. The 80 nm diameter particles are provided at 5 OD in a carbonate buffer (~2 mM). The 80 nm diameter particles are less stable at higher concentrations in the bare state and are provided at a 5 OD concentration. Conjugation protocols for both the 40 nm and 80 nm BioReady™ bare particles are available on nanoComposix's website.

Table 2. BioReady™ Bare Products.

Product	Concentration (OD)	Concentration (mg/mL)	Buffer
BioReady™ Bare 40 nm Citrate	20	0.8 – 0.95	0.02 mM citrate
BioReady™ Bare 40 nm Carbonate	20	0.8 – 0.95	Water
BioReady™ Bare 80 nm Carbonate	5	0.18 – 0.21	2.0 mM carbonate

Comparison Between Citrate and Carbonate Absorption Conjugates

To measure the difference in lateral flow assay sensitivity between the carbonate and citrate BioReady Bare particles, an anti-cardiac troponin I (anti-cTnI) antibody was adsorbed onto the surface of carbonate or citrate buffered BioReady Bare particles. After conjugation, the conjugate was washed three times in a purification buffer before concentrating to a final concentration of 20 OD/mL. The conjugates were sprayed down onto conjugate pads and assembled into strips. The assembled strips were run with various concentrations of cTnI and the test line intensity was read out using a Qiagen ESE Quant reader. The results are shown in Figure 6. The carbonate buffered BioReady Bare particles had an increased sensitivity compared to the citrate BioReady Bare particles. A number of our customers have reported similar results where the carbonate buffered particles have an increased sensitivity compared to citrate buffered gold particles.

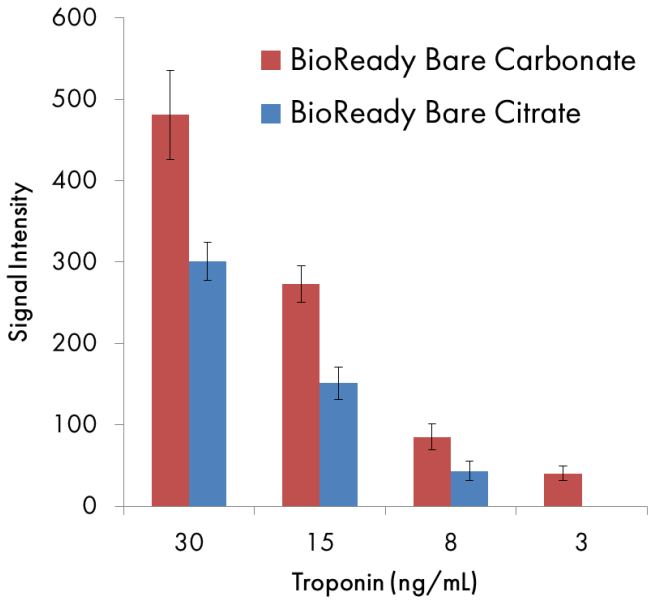

Figure 6. Signal intensity of test line in lateral flow strips using anti-cTnI conjugates on either carbonate buffered BioReady Bare particles or Citrate buffered BioReady Bare particles.

BioReady pH Adjustment

For successful conjugation pH and salt concentrations are often adjusted in order to maximize the efficacy of the antibody adsorbed to the particle surface. BioReady bare particles with a citrate surface are provided in a citrate buffer (0.02 mM sodium citrate). BioReady bare particles with a carbonate surface are provided unbuffered in deionized water. To adjust the pH of the starting solution the BioReady bare particles should be pH adjusted right before conjugation. At nanoComposix, we typically adjust the pH of the solution with solutions of 10 mM potassium phosphate monobasic (for pH ranges of 6.3 – 8.2) or 10 mM potassium phosphate dibasic (for pH ranges of 7.2 to 8.6). Table 3 below provides estimates of the amount of 10 mM poteassium phosphate basic/tribasic to add to a 5 mL sample of BioReady citrate/carbonate gold in order to reach the target pH. Due to the age of the solution and exposure to CO2 in the atmosphere, the pH of the BioReady gold solutions can

change with time. Titrations must be made with care to make sure that the addition of the buffers doesn't overshoot the target pH.

20 OD Citrate 40 nm Gold 5 mL Aliquot			20 OD Carbonate 40 nm Gold 5 mL Aliquot			5 OD Carbonate 80 nm Gold 5 mL Aliquot		
Target pH	Monobasic KH_2PO_4 (μL)	Dibasic KH_2PO_4 (μL)	Target pH	Monobasic KH_2PO_4 (μL)	Dibasic KH_2PO_4 (μL)	Target pH	Monobasic KH_2PO_4 (μL)	Tribasic KH_2PO_4 (μL)
6.4		0	6.4			6.4		
6.6		1	6.6			6.6		
6.8		2	6.8			6.8		1
7.0		4	7.0	0		7.0		2
7.2		7	7.2	1		7.2		3
7.4		9	7.4	5		7.4		6
7.6		16	7.6	14		7.6		14
7.8		24	7.8	28		7.8		24
8.0		43	8.0	49		8.0		40
8.2		91	8.2	63		8.2		72
8.4			8.4	113		8.4		136
8.6			8.6	233		8.6		256
8.8			8.8			8.8		656
9.0			9.0			9.0		1556

Table 3: Recommendations for pH adjustment of 20 OD citrate 40 nm gold, 20 OD carbonate 40 nm gold, and 5 OD carbonate 80 nm gold.

Lot Size and Pricing

The BioReady™ nanoparticles are fabricated within the ISO 13485 compliant quality system to produce high lot-to-lot consistency of the particle properties. Lot sizes as large as 400,000 OD-mLs (equivalent to 400 L of 1 OD gold) are possible. A large lot can generate up to 1,000,000 lateral flow assay strips. Specific lots can be reserved under a supply contract that allows for sampling from the same lot for up to a year. Using the same lot reduces downtime from re-optimization when switching to a new lot. The large lot manufacturing also allows the extensively characterized nanoparticles to be available at competitive pricing. When comparing pricing, it is important to adjust for prices that are supplied for different volumes and concentrations. Consider pricing in terms of OD-mL, which is the price of the product divided by the OD of the solution times the volume. For example, if 100 mL of gold nanoparticles at a 10 OD costs $600, then the cost per OD-mL is $600/(100*10) = $0.60. Contact nanoComposix for a quote or help with comparing costs.

Conclusion

The BioReady™ Bare gold maximizes sensitivity for physisorption conjugation. Some researchers fabricate gold nanoparticles in-house; however, the characterization that is necessary to ensure that each batch meets specification and the time lost in re-optimizing when using small volume lots adds significant costs. nanoComposix offers a three-lot evaluation kit for those interested in verifying that the bare gold particles can be a drop-in replacement for the gold nanoparticles currently in use.

Version 1.2
Date 2/8/2018