Technical Datasheet (TDS)

Gold Nanoparticles

Product Information

Product Name	20nm gold nanoparticles 30nm gold nanoparticles 40nm gold nanoparticles 50nm gold nanoparticles 60nm gold nanoparticles 70nm gold nanoparticles 80 nm gold nanoparticles
Catalog Number	WI-20 WI-30 WI-40 WI-50 WI-60 WI-70 WI-80
Form	Liquid
Concentration	100 OD in deionized water containing 0.02% BND.
Shelf life and storage	12 months if stored unopened at 2–8 °C, and should be used within 2 months after opening. Do not freeze.

Test Specifications

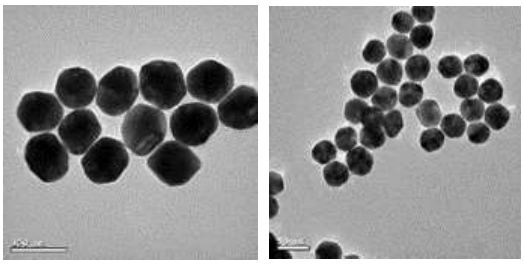
Test	Specification
Peak wavelength	WI-20: 515~519 nm by UV spectrophotometer WI-30: 520~522 nm by UV spectrophotometer WI-40: 523~526 nm by UV spectrophotometer WI-50: 528~532 nm by UV spectrophotometer WI-60: 534~539 nm by UV spectrophotometer WI-70: 540~544 nm by UV spectrophotometer WI-80: 545~548 nm by UV spectrophotometer
Optical density (OD)	1.0 +/- 0.05 OD at 1/100 dilution by UV spectrophotometer
Polydispersity Index (PDI)	＜0.2 by dynamic light scattering
Appearance (Color)	Visual inspection according to internal reference

Additional Information

Handling of gold nanoparticles	It is not recommended to pipette directly from the original bottle, as this can contaminate the bulk solution and cause gold aggregation. A better practice is to pour the required volume of the liquid into a clean container and pipette from there.

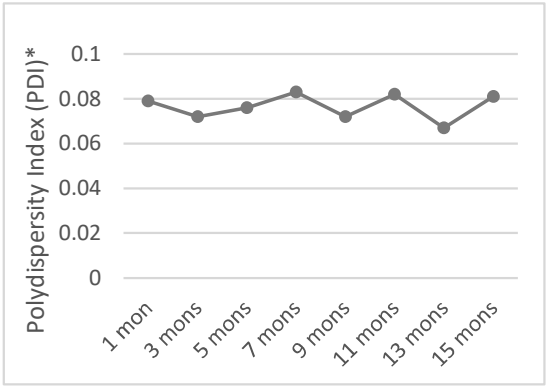
Technical Datasheet (TDS)

Product Data



TEM images of the gold nanoparticles

The gold nanoparticles (Left: WI-60, Right: WI-40) have a monodisperse nature, with a spherical and uniform shape.



Real-time stability evaluation of 50nm gold nanoparticles at 2-8°C

The gold nanoparticles (WI-50) were stored at 2-8°C for 15 months and periodically monitor the polydispersity index (PDI) value by dynamic light scattering (DLS).

The results indicate that the gold nanoparticles (WI-50) remain stable under recommended storage conditions (2–8 °C) after 15 months of storage.

* Polydispersity Index (PDI) is used to describe the non-uniformity of the particle size distribution. The smaller the PDI, the more homogeneous the nanoparticles.

Gold Nanoparticles

Introduction

Gold nanoparticles have been used as effective carriers in lateral flow assays to detect the target analytes in clinical samples. Due to their unique physical and chemical properties, colloidal gold can be easily conjugated with antibodies or antigens without altering their activity or specificity. High-quality gold materials are essential to deliver superior consistency and performance in rapid diagnostic tests.

The interaction of gold nanoparticles with proteins

Adsorption of proteins on the surface of gold nanoparticles is driven by three major forces: electrostatic interaction, hydrophobic binding, and dative bonding. Initially, the negatively charged gold particles will attract positively charged functional groups in proteins. As the proteins approach, hydrophobic patches within them bind to the hydrophobic areas of the gold particles through hydrophobic interactions. Additionally, proteins with sulfur-containing amino acid residues may form Au-S bonds with gold atoms. Therefore, the formation of gold nanoparticle-protein complexes is a complex process that depends on the characteristics of both the proteins and the gold nanoparticles. The shape, size, and surface chemistry of gold play important roles in this process.

Characteristics of gold nanoparticles

The physical properties of gold nanoparticles affect the efficiency and reproducibility of their conjugation with proteins, which, in turn, affect assay sensitivity, specificity, and manufacturing consistency. For lateral flow applications, the ideal gold nanoparticles should have the following attributes:

Monodispersity

Monodispersity is a key performance parameter for colloidal gold. Monodisperse gold nanoparticles have a uniform size and spherical shape. When a lateral flow test is run, gold conjugates, along with the clinical sample, will flow evenly from the conjugation pad onto the membrane. However, if the gold particles are polydisperse, with different sizes and irregular shapes, the larger gold conjugates will move slower than the smaller ones on the membrane. This difference in flow rate can cause variability in the test results.

Figure 1.
TEM images of monodisperse and polydisperse gold nanoparticles.

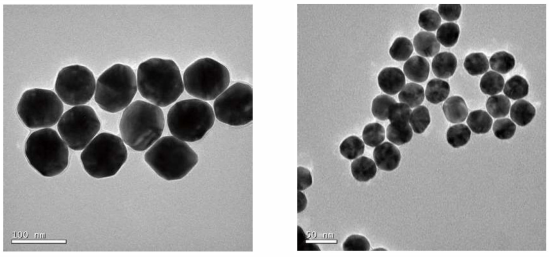


Figure 1a.
The monodisperse gold nanoparticles (Left: WI-60, Right: WI-40) with a consistent, spherical shape.

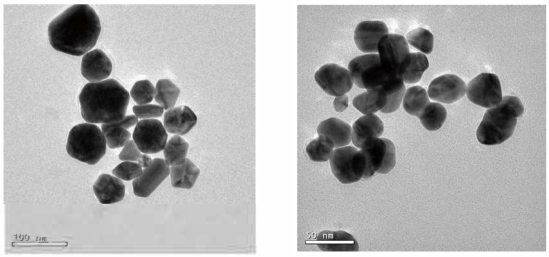


Figure 1b.
The polydisperse gold nanoparticles (Left: 60nm, Right: 40nm) with uneven sizes and irregular shapes.

Size

The size of gold nanoparticles directly affects the sensitivity of lateral flow assays. In sandwich immunoassays, larger gold nanoparticles (60nm & 70nm) can achieve higher assay sensitivity than smaller ones (30nm & 40nm).

Figure 2.
Sensitivity comparison of five different sizes of gold nanoparticles (WI-30, WI-40, WI-50, WI-60, WI-70) in procalcitonin (PCT) assay by testing human serum samples.

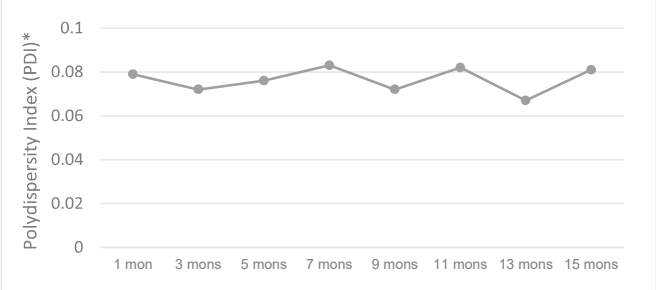
(A)30nm (WI-30) **(B)**40nm (WI-40) **(C)**50nm (WI-50)
(D)60nm (WI-60) **(E)**70nm (WI-70)

Colloidal stability

Gold nanoparticles are commonly synthesized by citrate reduction method. Due to the limitations of this method, the dispersion stability decreases when the size of colloidal gold reaches 50nm or larger. Poorly prepared gold nanoparticles tend to be polydisperse and may gradually aggregate over time.

Typically, high-quality gold nanoparticles have a shelf life of at least 12 months under appropriate conditions. It is recommended that manufacturers carry out stability studies to assess the changes in gold materials over extended periods of time before proceeding to the test strip production stage. An example of a long-term stability test is shown in Figure 3.

Figure 3.
Real-time stability evaluation of 50nm (WI-50) gold nanoparticles at 2-8°C *.



* Polydispersity Index (PDI) is used to describe the non-uniformity of the particle size distribution. The smaller the PDI, the more homogeneous the nanoparticles.

Concentration

High-concentration gold particles (> 50 OD) offer several advantages over conventional ones (1-5 OD) for lateral flow applications. Conjugating antibodies to gold particles at a high concentration could improve coupling efficiency, as it increases the chances of antibodies attaching to the surface of gold. Additionally, this approach could minimize the reaction volume in the gold conjugation procedure and save time and costs associated with centrifugation steps afterward.

Our gold products are supplied at a concentration of 100 OD, allowing kit manufacturers to perform antibody conjugation at 40 OD. This significantly reduces the cost of reagents (antibody or antigen) by up to two-thirds without sacrificing sensitivity, making it a cost-effective solution for lateral flow applications (see Table 1).

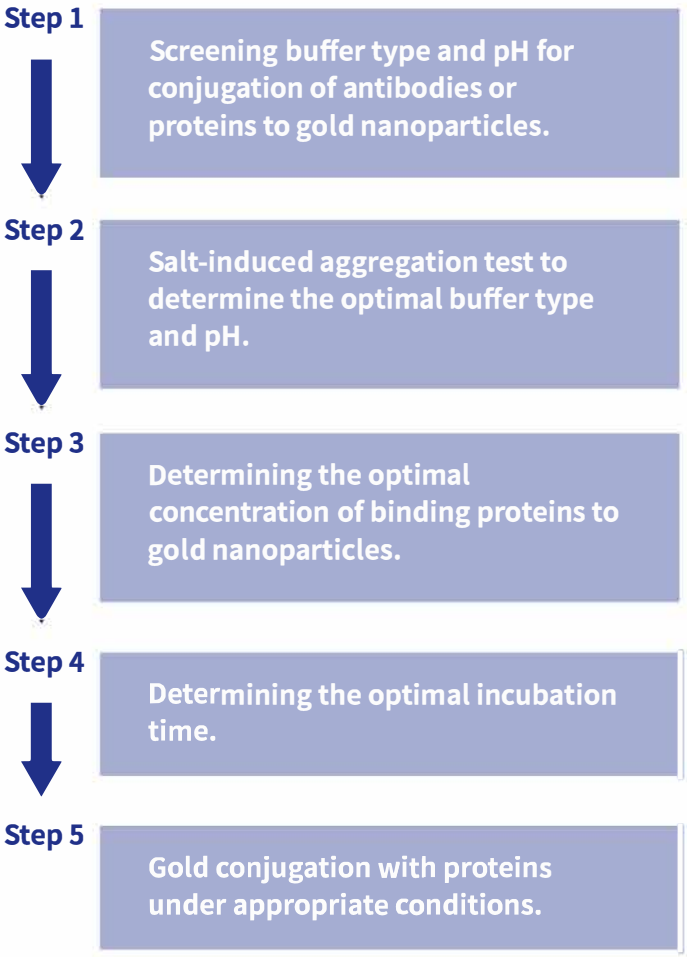
Table 1.
Comparison of gold conjugation using gold versus conventional gold.

Assay	SARS-Cov-2	
Production Batch	1M tests	
Gold Nanoparticles	gold	Conventional gold
Concentration	100 OD	1 OD
Conjugation Volume	1.7 L	68 L
Centrifugation Time	1~2 h	2 d
Antibody	100 mg~250 mg	>350 mg

Gold Conjugation Protocol

The protocol provides general guidelines for conjugating antibodies or proteins to gold nanoparticles. As proteins vary in their net charge and charge distribution, it is necessary to determine the optimal conjugation conditions, including buffer type, pH, protein concentration and incubation time, through a preliminary experiment before proceeding with the conjugation procedure.

Gold Conjugation Flow Chart



Reagents:

old nanoparticles at a concentration of 100 OD

Antibodies or proteins supplied at a concentration of
>1mg/mL

Gold conjugates storage buffer containing buffer salts, surfactants, sugars (sucrose or trehalose), and blocking proteins (BSA or Casein)

10% (w/v) NaCl solution

Buffer at the required pH (see Appendix 1)

Protocol

Step 1:
Screening buffer type and pH for conjugation

The most commonly used buffers for conjugation are listed below (Table 2). In our standard procedure, the coupling of proteins to gold nanoparticles is carried out in a set of unique buffers with different pH points. The optimal pH and buffer type are then determined by salt-induced aggregation test in step 2. The following is an example of coupling mouse monoclonal antibodies to gold nanoparticles under various buffer conditions.

Table 2.
Commonly used buffers for conjugation

Buffer Type	pH Range
0.01M Sodium citrate-citric acid buffer	3.0-6.6
0.01M Phosphate buffer	5.8-8.0
0.01M Borate Buffer	7.4-9.0
0.01M Citric acid- sodium tetraborate buffer	4.4-9.1

Procedure:

1. Add 60 µL of the following four buffers with the required pH to each of the four test tubes.
   A. 0.01M Phosphate buffer, pH 6.8
   B. 0.01M Phosphate buffer, pH 7.4
   C. 0.01M Borate buffer, pH 7.4
   D. 0.01M Borate buffer, pH 8.0

Note: As physical and chemical properties can vary significantly between proteins, we suggest experimenting with additional buffer types and pH for your proteins. A list of candidate buffer solutions is provided below (Table 3). Typically, the pH for conjugation should be slightly above the isoelectric point of the binding proteins. This is considered to be a starting point for determining of the appropriate pH. The optimal binding pH may require further fine-tuning using the selected buffer type.

Table 3.
A list of candidate buffer solutions for conjugation

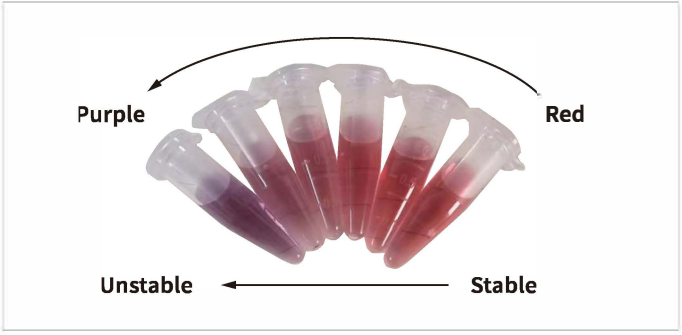
Buffer Type	pH
0.01M Sodium citrate-citric acid buffer	5.5
0.01M Phosphate buffer	6.0
0.01M Phosphate buffer	6.2
0.01M Borate buffer	8.4
0.01M Borate buffer	9.0
0.01M Carbonate-bicarbonate buffer	9.5

2. Label each tube with all pertinent information.
3. Vortex gold nanoparticles (100 OD) to ensure that the particles are completely suspended.
   Pipette 40 uL of gold nanoparticles (100 OD) into
4. each tube that contains the four selected buffer solutions with the required pH.
   Mix and thoroughly vortex the diluted gold nanoparticles (40 OD).
5. Add the appropriate amount of antibody to each labeled test tube. The typical concentration for
6. conjugation in step 1 is 60 ug/mL.
   React for 2 hours at room temperature with continuous mixing.
7. After conjugation, take a small aliquot (25 uL) of the gold conjugates (40 OD) for further salt-
8. induced aggregation test in step 2.

Step 2:
Salt-induced aggregation test *

The salt-induced aggregation test is a quick and simple method to monitor the colloidal stability of gold conjugates. Briefly, after conjugation, mix the gold conjugates with a NaCl solution and leave them at room temperature for 5 minutes. A color change from red to purple or blue indicates the aggregation of the gold conjugates (see Figure 4), suggesting that the buffer type and pH may not be suitable for your antibodies or proteins.

Figure 4. Salt-induce aggregation test



Procedure:

1. Add 975 uL of deionized water into each of the four clean test tubes.
2. Pipette 25 uL of gold conjugates (40 OD) into each test tube containing deionized water, to make a final volume of 1 mL (Note: The concentration of gold conjugates is now 1 OD). Label the tubes with all pertinent information.
3. Add 100 uL of 10% (w/v) NaCl solution to each test tube containing the diluted gold conjugates (1 OD).
4. Mix thoroughly and leave at room temperature for 5 minutes. Observe the color change of the gold conjugates. **
5. Read the results. Select the buffer type and pH that result in the best colloidal stability of gold conjugates.

* To determine the optimal buffer type and pH, the most accurate method is to validate the gold conjugates directly in functional test strips. Select the buffer type and pH that result in optimal sensitivity, specificity, and stability for your assays.
** Use 1 OD of gold nanoparticles as a color control. This can be done by diluting 100 OD of gold nanoparticles with deionized water.

Step 3:
Determining the optimal concentration of binding proteins to gold nanoparticles

1. Add 60 uL of the selected buffer with the required pH to each of the four test tubes.
2. Pipette 40 uL of gold nanoparticles (100 OD) into each tube and mix thoroughly.
3. Add the appropriate amount of antibody to each tube to achieve final concentrations of 40 ug/mL, 50 ug/mL, 60 ug/mL and 80 ug/mL, respectively. *
4. React for 2 hours at room temperature with continuous mixing.
5. Add 5 uL of 10% (w/v) BSA solution to the gold conjugates (40 OD). Mix thoroughly and leave at room temperature for 1h. **
6. After blocking, centrifuge at 2310 g for 10 min. The time and speed of the centrifugation depend on the size of gold nanoparticles and should be adjusted accordingly for optimal performance.

7. Remove the supernatant and resuspend gold conjugates to a final concentration of 40 OD in gold conjugates storage buffer.
8. Take an aliquot of gold conjugates (40 OD) for further functional testing. Select the antibody concentration that result in optimal sensitivity, specificity, and stability for your assays.

* The optimal concentration of the proteins needs to be determined experimentally for each assay.
** The optimal blocking agents need to be determined experimentally for each assay. BSA, Casein, or other commercial blockers are commonly used in lateral flow applications.

Step 4:
Determining the optimal incubation time

1. Add 60 uL of the selected buffer with the required pH to each of the four test tubes.
2. Pipette 40 uL of gold nanoparticles (100 OD) into each tube and mix thoroughly.
3. Add the appropriate amount of antibody determined in step 3 to each of the four test tubes.
4. React at room temperature for 30min, 60min, 90min and 120min, respectively.
5. After conjugation, take a small aliquot (25 uL) of gold conjugates (40 OD) for further salt-induced aggregation test in step 2.
6. Repeat step 2 to determine the optimal incubation time. *

* To determine the optimal incubation time, the most accurate method is to validate the gold conjugates directly in functional test strips. Select the incubation time that result in optimal sensitivity, specificity, and stability for your assays.

Step 5:
Gold conjugation with proteins under appropriate conditions

1. Add 60 uL of the selected buffer with the required pH to the test tube.
2. Pipette 40 uL of gold nanoparticles (100 OD) into the tube and mix thoroughly.
3. Add the appropriate amount of the antibody and react at room temperature for the optimal time determined in step 4.
4. Add 5 uL of 10% (w/v) BSA solution to the gold conjugates (40 OD). Mix thoroughly and leave at room temperature for 1h. *
5. After blocking, centrifuge at 2310 g for 10 min. The time and speed of the centrifugation depend on the size of gold nanoparticles and should be adjusted accordingly for optimal performance.
6. Remove the supernatant and resuspend gold conjugates to a final concentration of 40 OD in gold conjugates storage buffer.
7. Mix thoroughly and store at 4°C for further use.

* The optimal blocking agents need to be determined experimentally for each assay. BSA, Casein, or other commercial blockers are commonly used in lateral flow applications.

Appendix 1

Recipes for Commonly Used Buffers

The most commonly used buffers for conjugation are listed below. To prepare a buffer with a specific pH, varying amounts of solution A and B are mixed in the appropriate proportions provided in each table.

0.01M Sodium citrate - Citric acid Buffer

Solution A
0.01 M Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)
2.101 g/L (MW 210.14)

Solution B
0.01 M Sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)
2.941 g/L (MW 294.10)

pH	3.0	3.6	4.0	4.6	5.0	5.6	6.0	6.6
Solution A (mL)	18.6	14.9	13.1	10.3	8.2	5.5	3.8	1.4
Solution B (mL)	1.4	5.1	6.9	9.7	11.8	14.5	16.2	18.6

0.01M Phosphate Buffer

Solution A
0.01 M Sodium phosphate dibasic dihydrate
($Na_2HPO_4 \cdot 2H_2O$) 1.780 g/L (MW 177.99)

Solution B
0.01 M Sodium phosphate monobasic monohydrate
($NaH_2PO_4 \cdot H_2O$) 1.38 g/L (MW 137.99)

pH	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2
Solution A (mL)	8.0	12.3	18.5	26.5	37.5	49.0	61.0	72.0
Solution B (mL)	92.0	87.7	81.5	73.5	62.5	51.0	39.0	28.0

pH	7.4	7.6	7.8	8.0
Solution A (mL)	81.0	87.0	91.5	94.7
Solution B (mL)	19.0	13.0	8.5	5.3

0.01M Citric acid - Sodium tetraborate Buffer

Solution A *
0.005 M Sodium tetraborate decahydrate
($Na_2B_4O_7 \cdot 10H_2O$) 1.907 g/L (MW 381.37)

Solution B
0.01 M Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)
2.101 g/L (MW 210.14)

pH	4.4	4.9	5.5	6.0	7.8	8.4	8.8	9.1
Solution A (mL)	6.0	6.5	7.0	7.2	7.5	8.0	8.7	9.0
Solution B (mL)	4.0	3.5	3.0	2.8	2.5	2.0	1.3	1.0

* Keep container tightly sealed during storage.

0.02M Borate Buffer *

Solution A **
0.005 M Sodium tetraborate decahydrate
($Na_2B_4O_7 \cdot 10H_2O$) 1.907 g/L (MW 381.37)

Solution B
0.02 M Boric Acid (H_3BO_3)
1.237 g/L (MW 61.83)

pH	7.4	7.6	7.8	8.0	8.2	8.4	8.7	9.0
Solution A (mL)	1.0	1.5	2.0	3.0	3.5	4.5	6.0	8.0
Solution B (mL)	9.0	8.5	8.0	7.0	6.5	5.5	4.0	2.0

* To prepare a 0.01M borate buffer, dilute a 0.02M borate buffer with an equal volume of deionized water.
** Keep container tightly sealed during storage.

Appendix 2

Additional Information

Handling of gold nanoparticles

It is not recommended to pipette directly from the original bottle, as this can contaminate the bulk solution and cause gold aggregation. A better practice is to pour the required volume of the liquid into a clean container and pipette from there.

Storage conditions

The gold nanoparticles are shipped at ambient temperature. For long-term storage, it is recommended to keep them at 2-8°C. The product is stable for 18 months if stored unopened at 2-8°C, and should be used within 2 months after opening. Avoid freezing, as it may cause irreversible aggregation.

Gold Nanoparticles

ChemWhat

- Starter evaluation kit including a range of particle sizes from 20nm to 80nm for your R&D scientists.
- Large batch size to ensure continuous supply.
- Gold particles generate best in class performance for lateral flow applications.

	Nominal Diameter	λ_{max}	Pack Size*	Concentration	Batch Size*	Lead Time
WI-20	20nm	515~519nm	1ml, 10ml, 25ml, 100ml, 500ml	100 OD	8 L	2 weeks
WI-30	30nm	520~522nm	1ml, 10ml, 25ml, 100ml, 500ml	100 OD	8 L	2 weeks
WI-40	40nm	523-526nm	1ml, 10ml, 25ml, 100ml, 500ml	100 OD	12 L	2 weeks
WI-50	50nm	528-532nm	1ml, 10ml, 25ml, 100ml, 500ml	100 OD	12 L	2 weeks
WI-60	60nm	534-539nm	1ml, 10ml, 25ml, 100ml, 500ml	100 OD	12 L	2 weeks
WI-70	70nm	540-544nm	1ml, 10ml, 25ml, 100ml, 500ml	100 OD	12 L	2 weeks
WI-80	80nm	545~548nm	1ml, 10ml, 25ml, 100ml, 500ml	100 OD	12 L	2 weeks

*** 1L could supply the production of 3 million test strips.**

Gold Nanoparticles

ChemWhat

100 OD

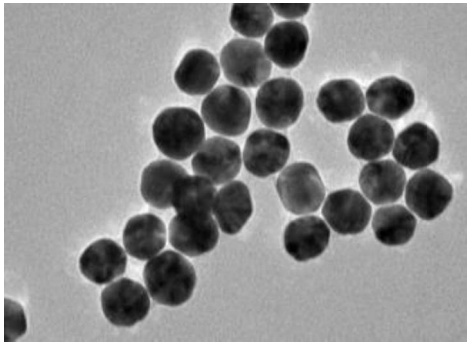
Gold products

1 OD

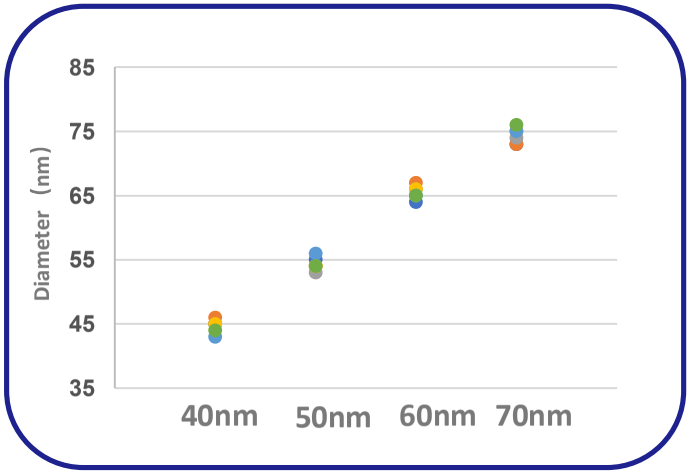
Traditional gold products

Advantages

- More than 1 billion test strips were manufactured with our gold nanoparticles during the COVID-19 pandemic.
- Larger sizes (60nm-80nm) to increase the assay sensitivity*.
- Monodisperse nature with spherical and uniform shape to ensure lot-to-lot consistency and assay specificity.
- Simplify gold conjugation process to save production time and equipment costs.
- 12 months shelf life at 2-8°C.



TEM images gold nanoparticles



Size distribution of gold nanoparticles from 6 lots

10/12

* Larger gold nanoparticles (60nm-80nm) exhibited equivalent sensitivity to 300nm latex beads from the leading competitor.

ChemWhat

“Simplify rapid kits manufacturing process with our innovative *Gold Nanoparticles*!”

1M Tests Production

1.7 L

Conjugation Volume

68 L

1.5 hours

Centrifugation

2 Days

200 mg

Antibody Consumption

> 350 mg

11/12

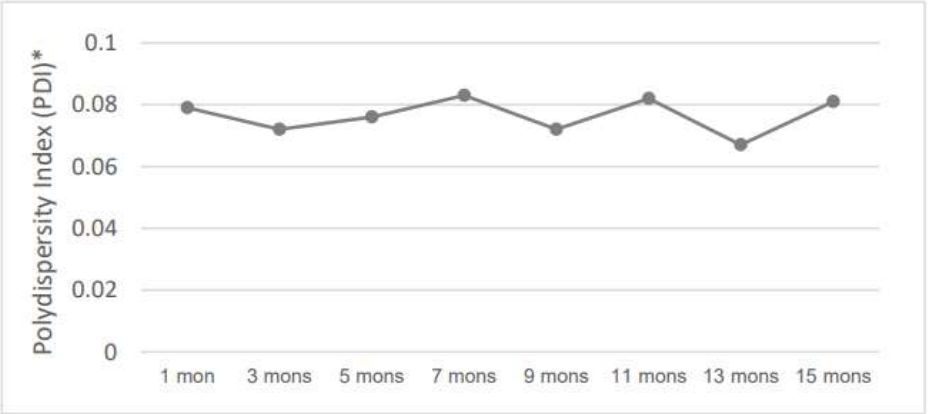
Performance Data

ChemWhat

Sensitivity comparison of five different sizes of gold nanoparticles

(A)30nm (WI-30) **(B)**40nm (WI-40) **(C)**50nm (WI-50)
(D)60nm (WI-60) **(E)**70nm (WI-70)

Real-time stability evaluation of 50nm (WI-50) gold nanoparticles at 2-8°C



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