Selenium Nanoparticles

Higher sensitivity particles

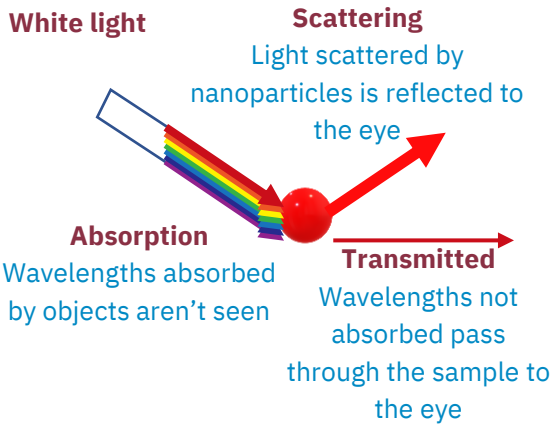
Passive Adsorption optimisation

Selenium nanoparticles (SeNP) for lateral flow assays

The bright red colour of large selenium colloid solutions makes them ideal for use as staining reagents to label low concentrations targets in immunoassays. Glantreo's Selenium nanoparticles (SeNP) offer an alternative to gold nanoparticles (AuNP) lateral flow antigen (LFA) tests.

SeNP achieve high sensitivities due to size, brightness, and stability at high concentrations

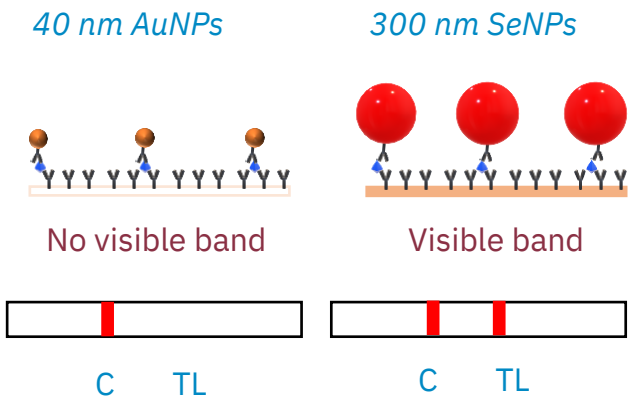
Unique Physio-optical properties



A simplified way of measuring and describing the visibility of a solution is by measuring its Extinction optical density (OD) by UV-Vis. Extinction OD is a sum of the spectra for light absorbed and scattered by an object. However, unlike smaller nanoparticles where colour is mostly governed by wavelengths absorbed, a considerable amount of the Extinction OD of large SeNP comes from scattered light, which makes it difficult to measure the colour that can be seen by eye.

Due to their larger size, 300nm SeNPs are more visible than 40nm AuNP to the naked eye. The increased diameter of 300nm SeNP produces a bigger optical signal per binding event, meaning at low concentrations of antigen, 300 nm SeNPs are more visible than their smaller counterparts.

Low concentration of analyte



Stable conjugates at OD30

Glantreo's SeNP are stable with adsorped antibodies up to OD30. This is a major advantage over gold nanoparticles (AuNP) which aggregate at high concentrations and for this reason are usually used at OD2. This means that SeNP conjugates can be applied to the test strip at OD30 and remain stable. The combination of bright, large size nanoparticles, combined with stability in a high concentration conjugate, mean that bright bands can be achieved for the test line with low concentrations of antigen present (pg/mL).

Recommended starting protocol for assay optimisation

Reagents

- Buffers with pH close to antibody of interests PI
- Antibody of interest
- Bovine Serum Albumin (30% in saline, protease free)
- BSA powder
- Sucrose powder
- 1M HCl
- Ultrapure water
- Sodium Tetraborate decahydrate
- 10% NaCl in ultrapure water

Equipment

- Pipettes with tips
- Eppendorf tubes
- Clean glass flasks
- pH meter
- Vortex or shaker
- UV-Vis Spectrophotometer
- Analytical balance
- Benchtop centrifuge

Selecting the best buffer for passive adsorption

Stable interactions between the antibody and SeNP are governed by pH. For the best binding to happen, the pH of the buffer should be close to the antibody PI.

Recommended buffers (0.1M-0.5M) to use with SeNP:

- MES buffer (pH 5.3)
- Sodium phosphate buffer (pH 5.7-7.3)
- Borate buffer (pH 7.7-9.3)

Titration of antibody concentration to determine minimum working concentration

Once buffer is selected, the minimal antibody concentration needed can be determined.

Titration procedure

1. Set up dilution series of chosen antibody between 0 and 1 mg in 25 uL total volume each. Dilute with 0.5% PBS.
2. Transfer 250 uL of SeNP colloid into 1.5 mL Eppendorf tubes for each concentration of antibody to be tested
3. Adjust the pH of the SeNP colloid to the isoelectric point of the antibody to be conjugated. The volume of buffer used is at a ratio of 1 in 20. For 250 uL of SeNP, use 12.5 uL of buffer
4. Add antibody to SeNP nanoparticles and mix well by pipette
5. Incubate for 15 minutes at room temperature, for best results use a method of gentle agitation such as a rotator
6. Add 10% NaCl solution at a 1:1 ratio. For example, add 50 uL 10% NaCl to 50 uL of pH adjusted SeNP and antibody solution, mix by pipette and incubate.
7. Observe samples for aggregation after 15 minutes, fully saturated samples will remain in solution with a bright red colour. Non optimum conditions will aggregate out of solution forming a precipitate.

Antibody adsorption procedure

First prepare the blocking buffer and drying buffer, scale according to volumes needed

Blocking buffer (30mL)

REAGENT	VOLUME (ML)
ULTRPURE WATER	**30 mL**
BSA IN 30% SALINE SOLUTION	**0.15 mL**

Drying buffer (10mL)

REAGENT	VOLUME (ML) WEIGHT (G)
ULTRPURE WATER	**6 mL**
SODIUM TETRABORATE DECAHYDRATE	**0.0126 g**
BSA (POWDER)	**0.2 g**
HYDROCHLORIC ACID (1M)	**By drop to adjust pH to 7.8**
SUCROSE (POWDER)	**0.33 g**
TWEEN 20 (SOLUTION)	**0.033 g**
ULTRAPURE WATER	**To make up to 10 mL final volume**

Prior to starting make sure SeNP colloid and antibody are well dispersed, and buffers have fully dissolved.

Preparing the Selenium antibody conjugate

Depending on the volume of product and optimum antibody concentration determined, the following can be scaled.

Steps

1. Combine buffer and antibody in clean container, seal container and gently shake for 5 mins at room temperature.
2. Ensure SeNP are well dispersed by gentle agitation for 1 minute before using. Add the correct amount of SeNP to the buffer and antibody solution. Gently pipette solution to mix.
3. Cover container and gently shake for 1 hr at room temperature $\leq$750rpm.

For 10mL of antibody conjugate, using a 1 mg/mL antibody stock solution, to achieve a 0.1mg/mL final solution

REAGENT	VOLUME (ML)
CHOSEN BUFFER	**0.5 mL**
CHOSEN ANTIBODY (1MG/ML)	**1 mL**
SELENIUM NANOPARTICLES	**8.5 mL**

4. After shaking, add 20 mL of blocking solution into container, seal container and shake for a further 45 minutes at room temperature $\leq$750rpm.

Centrifugation steps

Selenium nanoparticles are more sensitive than gold nanoparticles due to their size and material, therefore keep to lower centrifugation speeds and time to avoid aggregation

If scaling up this procedure, or pellet not forming, centrifuge for 1 hr

1. Add 10 mL of ultrapure water to the dispersion and stir for 5 minutes, then centrifuge for 30 mins at 200g to form a pellet.
2. Carefully remove the supernatant and add 30 mL of ultrapure water.
3. Repeat steps 1-2 above.
4. Carefully remove the supernatant and add 3.4 mL of drying buffer and carefully resuspend the pellet.
5. Check the OD of the SeNP antibody conjugate using a spectrophotometer. Dilute 1 in 20, and measure the peak absorbance, normalise using measurement at 1000nm (1100nm is used for bare SeNP), and multiply by dilution factor to calculate OD.
6. Adjust to desired OD using drying buffer.
7. Store in fridge between 2-8^{o}C until use.

Additional information for designing your lateral flow assay

- Glantreo's SeNP are larger than standard AuNP used in lateral flow and therefore require membranes with larger pore sizes :

UniSart, CN95 (Sartorius)

Unisart CN150 (Sartorius)

HI-Flow™ Plus HF090 (Merck)

HI-Flow™ Plus HF135 (Merck)

- Membranes with slower flow rates are better for higher sensitivity assays as they allow more time for nanoparticle antibody conjugates to bind at the test line.
- An advantage of SeNP is the stability to exist in conjugates in concentrations up to OD30, this allows for a brighter test line to be produced at low concentrations of antigen of interest.
- Optimisation of the number of nanoparticles used is also important. As these particles are larger, they can produce a bigger signal from binding. However, too higher concentration will result in steric effects reducing the number of particles that can bind and stay on the test line. Additionally, there may be bleed effects resulting from none specific binding if too high concentration is used. The number of nanoparticles/mL should also be titrated to achieve a balance between optimal signal and clear background.

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