AURION GOLD NANOPARTICLES - CARBOXYL FUNCTIONALIZED -

PRODUCT INFORMATION

Aurion Carboxyl-functionalized Gold Nanoparticles are polyethylene glycol-carboxyl stabilized gold nanoparticle solutions based on defined particle sizes of 6, 10, 15 and 25nm. Aurion nanoparticles are monodisperse, with a coefficient of variance <15%, but in most cases <10%.
The carboxylic end group is available for covalent binding to (macro)molecules such as peptides, proteins or other biomolecules having (a) primary amine(s).

Aurion Carboxyl-functionalized Gold Nanoparticles are free from protein and essentially free from salts.

A general conjugation procedure is described in this package insert.

Aurion Carboxyl-functionalized Gold Nanoparticles are supplied at an OD_{520nm} = 1 in a volume of 20 ml and should be stored at 4°C.
Do not freeze!

Aurion Gold Nanoparticles,- Carboxyl Functionalized - have a guaranteed shelf life of 12 months from the date of quality control analysis.

GENERAL REMARKS

Ligands need to contain one or more (exposed) primary amino groups to be suitable for covalent conjugation to Aurion Carboxyl-functionalized Gold Nanoparticles. Primary amines are present in e.g. the N-terminal side of peptides and in the side group of the amino acid lysine.

The conjugation relies on well known and proven EDC/sulfo-NHS chemistry. EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) is a water soluble carbodiimide which transforms the carboxyl groups on the gold to an active ester in the presence of sulfo-NHS (N-Hydroxysulfosucciimide, sodium salt). These sulfo-NHS esters are relatively stable in acidic environment and couple rapidly to the amine(s) in the target molecules. We recommend an activation pH of 5.0.

Ligands preferably have a concentration >1mg/ml. They need to be purified and free from other molecules containing primary amines. Amine- and carboxylate-free buffers should be used during the activation and conjugation process.

When covalently conjugating proteins suggested conjugation pH is 0.5 to 1 pH unit below the isoelectric point of the protein. For other ligands the optimum pH should be determined experimentally.

To obtain maximum conjugation to the particle surface, the amount of ligand required for conjugation is in general between 1 and 10x the calculated amount needed for full coverage. Optimal conjugation conditions may vary and need to be optimized experimentally.

Detailed background information on EDC/sulfo-NHS chemistry and covalent conjugation to carboxyl groups can be found in e.g., G.T. Hermanson, Bioconjugate Techniques 3rd edition, 2013 Academic Press.

INSTRUCTIONS FOR USE

MATERIALS

1. Aurion Carboxyl-functionalized Gold Nanoparticles
2. Amicon Ultra-4 30K (and 100 K) filter units, Millipore
3. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)
4. N-Hydroxysulfosucciimide, sodium salt (sulfo-NHS)
5. 2-Morpholinoethanesulfonic acid Monohydrate (MES)

All chemicals are reagent grade

CARBOXYL ACTIVATION

1. Prepare a 10mM MES buffer and adjust the pH to 5.0 with NaOH
2. Wash the Ultra-4 30K filter units with deionized water
3. Bring the requested volume of Aurion Carboxyl-functionalized Gold Nanoparticles in the filter unit(s)
4. Centrifuge at $1500xg_{av}$ for 10 minutes
5. Remove the solvent from the tube
6. Add 3ml of 10mM MES buffer, pH 5.0 per filter unit, mix and centrifuge at $1500xg_{av}$ for 15 minutes
7. Remove the concentrated Aurion Carboxyl-functionalized Gold Nanoparticles from the filter units and bring to the original sample volume with 10mM MES buffer, pH 5.0
8. Immediately before use, prepare a 100mM EDC solution in 10mM MES buffer, pH 5.0 (19.2mg EDC in 1 ml of MES buffer)
9. Add 20μl of 100mM EDC in MES buffer per ml of Aurion Carboxyl-functionalized Gold Nanoparticles and mix thoroughly
10. Incubate for 5 minutes
11. Immediately before use, prepare a 100mM sulfo-NHS solution in MES buffer, pH 5.0 (21.7mg in 1 ml of MES buffer)
12. Add 20 μl of sulfo-NHS solution per ml of Aurion Carboxyl-functionalized Gold Nanoparticles and incubate for 30 minutes
13. The Aurion Carboxyl-functionalized Gold Nanoparticles are now activated. Conjugation should follow immediately to avoid deactivation

CONJUGATION AND PURIFICATION

1. Adjust the ligand concentration preferably to 1mg/ml or higher and at bring to the desired conjugation pH.
2. Adjust the pH of the activated Carboxyl-functionalized Gold Nanoparticles to the conjugation pH with NaOH/HCl or make a buffer exchange using the Ultra-4 30K filter units. If you change to another buffer use one that does not contain amines and/or carboxyl groups. Preferred buffer concentration is 10mM.
3. As a guideline the ligand concentration in the conjugation mixture is preferably between $1x10^{-4}$ M and $1x10^{-5}$ M. After adding the ligand stir for 2 hours
4. Block excess of activated carboxyl groups with glycine or Tris(hydroxymethyl)aminoethane in a final concentration of 50mM, for 15 minutes
5. Centrifuge at $1500xg_{av}$ for 10 minutes using the 100 KDa Ultra-4 filter units

In case the MW of the ligand that is conjugated exceeds 100 kDa free ligand should be removed using ultracentrifugation acc. to the data mentioned in Table 1

Table 1:

Particle diameter	g-force	time
6 nm	$45,000xg_{av}$	45 minutes
10 nm	$45,000xg_{av}$	30 minutes
15 nm	$12,000xg_{av}$	45 minutes
25 nm	$12,000xg_{av}$	30 minutes

The pellets are partly solid and partly fluffy. The fluffy parts are collected and used for further purification. The solid pellets are not recovered as they are largely composed of aggregates. Non-conjugated ligands remain in the supernatant.

6. Repeat this step 2x
7. Collect the concentrated conjugate
8. Dilute in a preferred storage buffer, e.g, bring to OD_{520nm} = 1.0 using PBS + 0.1% BSA + 15mM NaN_3, pH 7.4

FURTHER PURIFICATION FOR ELECTRON MICROSCOPY

The collected fluffy pellets may be further purified using a 10-30% sucrose or glycerol density gradient ultracentrifugation step. This will result in increased particle size homogeneity as well as the removal of clusters, qualities that particularly benefit electron microscopy applications.
After centrifugation the supernatant on top of the gradient is removed and discarded. The upper third of the gradient is collected. If necessary the density gradient materal is removed by dialysis against PBS with 0.1% BSA + 15 mM NaN_3.

Table 2 summarizes gradient centrifugation data depending upon the particle diameter.

Table 2:

Particle diameter	g-force	time
6 nm	125,000xg_{av}	45 minutes
10 nm	50,000xg_{av}	45 minutes
15 nm	15,000xg_{av}	45 minutes
25 nm	10,000xg_{av}	45 minutes

After purification the gold conjugate OD_{520nm} is determined. The gold conjugate may be diluted to a desired optical density using PBS + 0.1% BSA + 15mM NaN_3, pH 7.4. The gold conjugate is stored at 4°C. Addition of glycerol or sucrose to a final concentration of at least 20% or 2M respectively allows freezing of the gold conjugates in liquid nitrogen and storage at -80 or -20°C (Slot and Geuze, *J. Cell Biol.* , 90, (1981), 533)

EXAMPLE

Conjugation of Biotin Hydrazide to Aurion Carboxyl-functionalized 10 nm Gold Nanoparticles

- Prepare a 10mM MES buffer and adjust pH to 5.0 with NaOH
- Prepare a stock solution of 100mg/ml of Biotin Hydrazide (e.g., 21339 Thermo Scientific) in Dimethyl Sulfoxide
- Wash 2 Ultra-4 filter units with deionized water
- Bring 2ml of Aurion 410.133 Carboxyl-functionalized 10nm Gold Nanoparticles in each of the 2 Ultra-4 units.
- Centrifuge at 1500xg_{av} for 10 minutes
- Add 3ml of 10mM MES buffer, pH 5.0 per filter unit, mix and centrifuge at 1500xg_{av} for 15 minutes
- Remove the concentrated Aurion Carboxyl-functionalized Gold Nanoparticles from the filter units and adjust the collected volume to 4ml with 10mM MES buffer, pH 5.0
- Immediately before use, prepare a 100mM EDC solution in 10mM MES buffer, pH 5.0
- Add 80 µl of 100mM EDC in MES buffer to 4ml of Aurion Carboxyl-functionalized Gold Nanoparticles and mix thoroughly
- Incubate for 5 minutes
- Immediately before use, prepare a 100mM sulfo-NHS solution in MES buffer, pH 5.0
- Add 80 µl and incubate for 30 minutes
- Add 20 µl of Biotin Hydrazide stock solution and incubate for 2 hours
- Block excess of activated carboxyl groups with glycine in a final concentration of 50mM, for 15 minutes
- Centrifuge at 1500xg_{av} for 10 minutes using the Ultra-4 filter units
- Repeat this step 2x
- Collect the concentrated biotinylated 10nm gold nanoparticles
  Dilute to OD_{520nm} = 1.0 using PBS + 0.1% BSA + 15mM NaN_3, pH 7.4

Activity of the biotinylated gold nanoparticles can be tested using a dot-spot test. Briefly, a dilution series of biotinylated albumin (highest concentration 1mg/ml) is spotted on a strip of nitrocellulose membrane. After washing and blocking, the strip is incubated with streptavidin in a concentration of 5 µg/ml. Strip is washed and incubated with the biotinylated gold conjugate diluted to OD_{520nm} = 0.1.
Detailed information on the dot-spot test can be found in Newsletter nr 4.

HOW TO ORDER

406.133: Gold Nanoparticles 6nm -Carboxyl Functionalized-, 4x5ml
410.133: Gold Nanoparticles 10nm -Carboxyl Functionalized-, 4x5ml
415.133: Gold Nanoparticles 15nm -Carboxyl Functionalized-, 4x5ml
425.133: Gold Nanoparticles 25nm -Carboxyl Functionalized-, 4x5ml

AUXILIARY PRODUCTS

For the conjugation of high MW macromolecules, e.g., antibodies, we recommend to conjugate via adsorption. AURION has a full range of Non-functionalized nanoparticles available for this purpose:

406.011: Gold Nanoparticlesl 6nm, 100ml
410.011: Gold Nanoparticles 10nm, 100ml
415.011: Gold Nanoparticles 15nm, 100ml
425.011: Gold Nanoparticles 25nm, 100ml

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