Gold Conjugation Kit (Thiolated Oligonucleotides) Protocol

Reduction of thiol-modified oligonucleotides

1. Prepare a 0.15 M sodium phosphate buffer, pH 8.5 supplemented with 0.1 M DTT.

Note: pH is important for proper reduction of oligonucleotide.

2. Dissolve lyophilized oligonucleotide to a final concentration of 500 µM in H_2O.
3. Mix 50 µL of dissolved oligonucleotide with 450 µL sodium phosphate buffer.
4. Incubate 1-2 hours at room temperature to reduce oligonuclotide.
5. Separate reduced oligonucleotide from trityl-SH and DTT using a NAP 5 column operated in H_2O, GE Healthcare.
6. Final eluate from NAP 5 column will be 1 mL in H2O with an approximate concentration of 25 µM.

Note: Exact concentration of final eluate can be measured with UV-VIS spectroscopy by measuring the absorbance at 260 nm.

Conjugation Procedure

Resuspend one vial of lyophilized gold nanoparticle with 740 µL of H_2O.

1. Transfer into a 1.5 mL microcentrifuge tube.
2. Add 160 µL of reduced thiolated oligonucleotide (25 µM (0.025 nmol/µL) in H_2O) as prepared above.

Note: The resulting number of oligonucleotides per particle can be lowered by reducing the amount of oligonucleotide stock solution added in this step. If you are reducing the volume of oligonucleotide stock solution added, compensate with H_2O to keep the volume the same in this step..

3. Add 100 µL of 1M NaCl.
4. Incubate for at least 1 hour at room temperature to allow binding of the oligonucleotide to the gold surface.

Note: Longer incubation times may improve surface coverage.

5. Centrifuge at the appropriate speed for your particular gold nanoparticle size for 30 minutes to pellet your oligonucleotide gold conjugate.
6. Remove supernatant.

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7. Resuspend conjugate in 200 µL of storage buffer. The optical density of the particles should be 10 if a 100% recovery is achieved.

Common storage buffer: 10 mM sodium phosphate buffer, pH 7.0, 100 mM NaCl and 0.01% (w/v) NaN_3.

8. Measure optical density with a spectrophotometer and adjust concentration as desired.
9. Store conjugate at 4°C

Suggested Centrifugation Conditions

Appropriate G forces for centrifugation of gold nanoparticles. Listed conditions are for a volume of 1 mL and centrifugation using a microcentrifuge, except for 5 nm gold nanoparticles that requires an ultracentrifuge.

Size	Centrifugation Force	Time
5 nm	100 kDa MWC Spin Column	30 min
10 nm	17000 x g	60 min (~50% recovery)
15 nm	17000 x g	30 min
20 nm	6500 x g	30 min
30 nm	4500 x g	30 min
40 nm	2500 x g	30 min
50 nm	2000 x g	30 min
60 nm	1125 x g	30 min
80 nm	600 x g	30 min
100 nm	400 x g	30 min
150 nm	180 x g	30 min
200 nm	100 x g	30 min

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