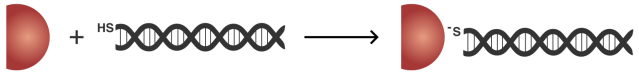
CYTODIAGNOSTICS

PRODUCT DATA SHEET

OligoREADY™ Gold Nanoparticle Conjugation Kit

Oligonucleotides



Description

Cytodiagnostics OligoREADY™ gold conjugation kits have been optimized for high efficiency one-step conjugation of thiolated oligonucleotides directly to the gold surface.

The kit contains ready-to-use pre-made mixtures. No activation, manipulation, or time consuming "salt-aging" steps are required for conjugation. Simply mix your reduced thiol-modified oligonucleotide with the supplied pre-activated gold nanoparticles. Conjugation of the oligonucleotide is achieved by the formation of a strong and stable gold-thiol bond without any additional linkers.

Features & Benefits

- Allows conjugation of oligonucleotides to gold nanoparticles with sizes between 5nm-100nm.
- Fast and convenient one-step conjugation reaction with no pre-activation requirements or manipulation of the gold nanoparticles.
- No time-consuming "salt-aging" procedures.
- Results in a thiol-oligo conjugated directly to the gold surface without any linkers.
- Optimized for use in crosslinking based lateral flow applications.

Gold Nanoparticle Specifications

Gold surface: Proprietary OligoREADY™-coating
Core diameter: Available with diameters from 5nm-100nm
Optical density (OD): OD=2 when the contents of each vial is dissolved to a final volume of 1 ml.
Particles per ml: Core size dependant, please see table II.
Lambda max: Core size dependant, please see table II.

Storage

Store at -20° C. Stable for at least 3 months if stored as specified.

Product Safety and Handling

This product is for R&D use only, not for drug, household, or other uses. Please review the material safety datasheet (MSDS) available online for proper safety and handling procedures.

Procedure

Reduction of thiol-modified oligonucleotides (e.g. trityl-S-S-Oligo)

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 oligonucleotide.

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 1ml in H_2O 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 260nm.*

Conjugation of thiolated oligonucleotide to OligoREADY™ gold nanoparticles

1. Resuspend one vial of lyophilized OligoREADY™ gold nanoparticle with 740 μl of H_2O.

2. Transfer into a 1.5 ml microcentrifuge tube.

Canada, Europe, Asia, Pacific and Africa
919 Fraser Drive, Unit 11, Burlington, ON Canada L7L 4X8
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics.com

United States, Mexico, South and Central America
5867 South Garnett Road, Tulsa, Oklahoma 74146 USA
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics-us.com

3. Add 160 µl of reduced thiolated oligonucleotide at 7.5 µM (0.0075 nmol/µl)* in H_2O as prepared above and incubate for at least 1 hour at room temperature.

***Note:** 7.5 µM oligonucleotide is a good starting concentration, but if aggregation or poor sensitivity is observed, the following oligonucleotide concentrations can be attempted for a given particle size range (based on a 30nt oligonucleotide):*

Particle size (nm)	5	10-20	30-100
[oligonucleotide] (µM)	5-50	5-25	5-15

4. Add 100 µl of 1M NaCl.

5. 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.*

6. Centrifuge at the appropriate speed for your particular gold nanoparticle size (see table I) for 30 minutes to pellet your oligonucleotide gold conjugate.

7. Remove supernatant.

8. Resuspend conjugate in 200 µl of storage buffer. The optical density of the particles should be 10 if a 100% recovery has been achieved.

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

9. Measure optical density with a spectrophotometer and adjust concentration as desired.

10. Store conjugate at +4°C

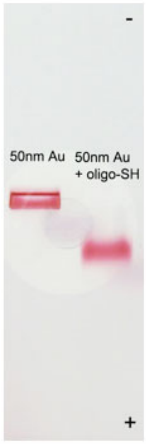


***Figure 1.** 0.5% (w/v) agarose gel analysis of 50nm OligoREADY™ gold nanoparticles before and after functionalization with a thiolated oligonucleotide (20 bases). Gel was operated at 100V in 0.5X TBE buffer for 30 minutes.*

***Table I.** Appropriate G forces for centrifugation of gold nanoparticles. Note that recommended conditions are for a volume of 1ml and centrifugation using a microcentrifuge, except for 5nm gold nanoparticles that require an ultracentrifuge.*

Size (nm)	Speed (*g*)	Time (min)
5	100,000	30
10	17,000	60 (~50% recovery)
15	17,000	30
20	6,500	30
30	4,500	30
40	2,500	30
50	2,000	30
60	1,125	30
80	600	30
100	400	30

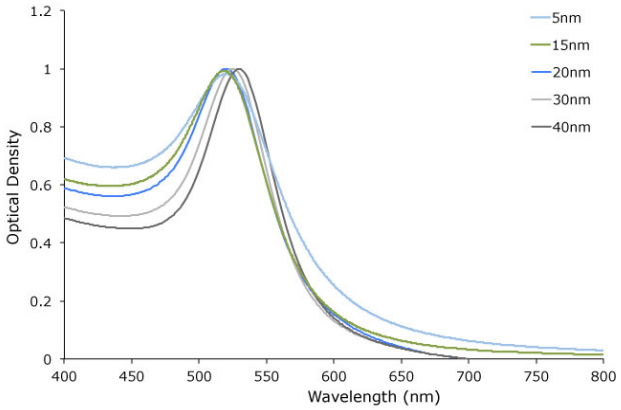
Canada, Europe, Asia, Pacific and Africa
919 Fraser Drive, Unit 11, Burlington, ON Canada L7L 4X8
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics.com

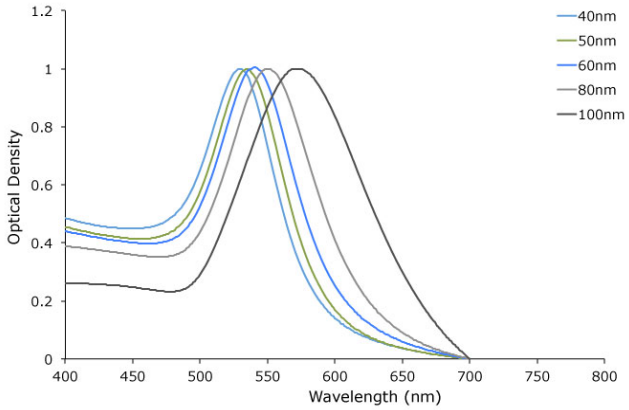
United States, Mexico, South and Central America
5867 South Garnett Road, Tulsa, Oklahoma 74146 USA
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics-us.com

CYTODIAGNOSTICS

Table II. *Gold nanoparticle specifications by size. Please note that all values below are indicated at an optical density of 1 (OD/cm^{-1}) at their respective lambda max. At other optical densities the values needs to be adjusted (e.g. NPS/ml (@OD2) = 2 x NPS/ml (@OD1)).*

Diameter (nm)	Peak SPR Wavelength (nm)	NPS/ml	Wt. Conc. (mg/ml)	Molar Ext ($M^{-1}cm^{-1}$)	Size Dispersity (+/-nm)	Particle Volume (nm^3)	Surface Area (nm^2)	Surface/ Volume Ratio	Particle Mass (g)	Molar Mass (g/mol)	Molar Conc.
5	515-520	5.47E+13	6.94E-02	1.10E+07	<15%	6.54E+01	7.85E+01	1.2	1.27E-18	7.64E+05	9.08E-08
10	515-520	5.98E+12	6.07E-02	1.01E+08	<15%	5.24E+02	3.14E+02	0.6	1.02E-17	6.11E+06	9.93E-09
15	520	1.64E+12	5.61E-02	3.67E+08	<12%	1.77E+03	7.07E+02	0.4	3.43E-17	2.06E+07	2.72E-09
20	524	6.54E+11	5.31E-02	9.21E+08	<12%	4.19E+03	1.26E+03	0.3	8.12E-17	4.89E+07	1.09E-09
30	526	1.79E+11	4.91E-02	3.36E+09	<12%	1.41E+04	2.83E+03	0.2	2.74E-16	1.65E+08	2.98E-10
40	530	7.15E+10	4.65E-02	8.42E+09	<12%	3.35E+04	5.03E+03	0.15	6.50E-16	3.91E+08	1.19E-10
50	535	3.51E+10	4.45E-02	1.72E+10	<10%	6.54E+04	7.85E+03	0.12	1.27E-15	7.64E+08	5.83E-11
60	540	1.96E+10	4.30E-02	3.07E+10	<10%	1.13E+05	1.13E+04	0.1	2.19E-15	1.32E+09	3.25E-11
70	548	1.20E+10	4.17E-02	5.03E+10	<10%	1.80E+05	1.54E+04	0.086	3.48E-15	2.10E+09	1.99E-11
80	553	7.82E+09	4.06E-02	7.70E+10	<10%	2.68E+05	2.01E+04	0.075	5.20E-15	3.13E+09	1.30E-11
90	564	5.37E+09	3.97E-02	1.12E+11	<8%	3.82E+05	2.54E+04	0.067	7.40E-15	4.46E+09	8.92E-12
100	572	3.84E+09	3.89E-02	1.57E+11	<8%	5.24E+05	3.14E+04	0.06	1.02E-14	6.11E+09	6.37E-12





Canada, Europe, Asia, Pacific and Africa
919 Fraser Drive, Unit 11, Burlington, ON Canada L7L 4X8
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics.com

United States, Mexico, South and Central America
5867 South Garnett Road, Tulsa, Oklahoma 74146 USA
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics-us.com

CYTO DIAGNOSTICS

Catalogue Number	Description	Sizes
OGC-5-X*	5nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-10-X*	10nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-15-X*	15nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-20-X*	20nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-30-X*	30nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-40-X*	40nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-50-X*	50nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-60-X*	60nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-70-X*	70nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-80-X*	80nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-90-X*	90nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions
OGC-100-X*	100nm OligoREADY Gold Nanoparticle Conjugation Kit	3 reactions & 10 reactions

*X Indicates quantity, i.e. -1 for a 3 reaction kit and -2 for a 10 reaction kit
For custom sizes and information on bulk quantities and prices please contact our customer service department.

Ordering Information

For ordering call 866-344-3954 or visit us online.

Canada, Europe, Asia, Pacific and Africa
919 Fraser Drive, Unit 11, Burlington, ON Canada L7L 4X8
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics.com

United States, Mexico, South and Central America
5867 South Garnett Road, Tulsa, Oklahoma 74146 USA
Tel: 866-344-3954 Fax: 289-204-9100
www.cytodiagnostics-us.com