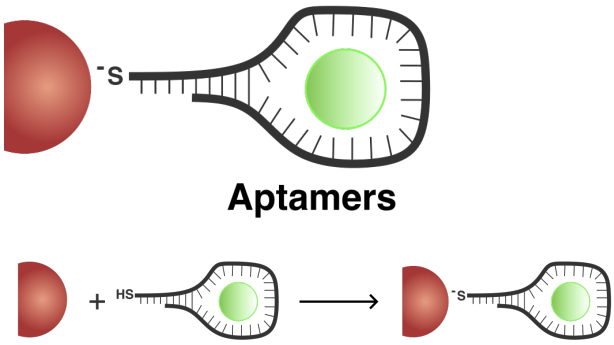
CYTODIAGNOSTICS

PRODUCT DATA SHEET

AptamerREADY™ Gold Nanoparticle Conjugation Kit



Description

Cytodiagnostics AptamerREADY™ gold conjugation kits have been optimized for high efficiency one-step conjugation of thiolated aptamers 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 aptamer with the supplied pre-activated gold nanoparticles. Conjugation of the aptamer is achieved by the formation of a strong and stable gold-thiol bond without any additional linkers.

Features & Benefits

- Allows conjugation of aptamers 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-aptamer conjugated directly to the gold surface without any linkers.
- Optimized for use in aptamer or aptamer/antibody based lateral flow applications.

Gold Nanoparticle Specifications

Gold surface: Proprietary AptamerREADY™-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 aptamers (e.g. trityl-S-S-aptamer)

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 aptamer.

2. Dissolve lyophilized aptamer to a final concentration of 500 μM in H_2O.

3. Mix 50 μl of dissolved aptamer with 450 μl sodium phosphate buffer.

4. Incubate 1-2 hours at room temperature to reduce aptamer.

5. Separate reduced aptamer 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 aptamer to AptamerREADY™ gold nanoparticles

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

2. Transfer into a 1.5 ml microcentrifuge tube.

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3. Add 160 µl of reduced thiolated aptamer 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 aptamer is a good starting concentration, but if aggregation or poor sensitivity is observed, the following aptamer concentrations can be attempted for a given particle size range (based on a 30nt aptamer):*

Particle size (nm)	5	10	15-70	80-100
[aptamer] (µM)	5-50	1-10	5-15	1-10

4. Add 100 µl of 1M NaCl.

5. Incubate for at least 1 hour at room temperature to allow binding of the aptamer 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 aptamer 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

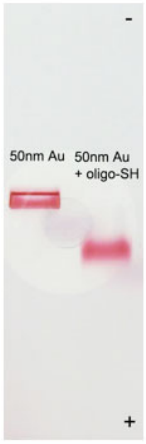

***Figure* I.** *0.5% (w/v) agarose gel analysis of 50nm AptamerREADY™ gold nanoparticles before and after functionalization with a thiolated aptamer (20 bases). Gel was operated at 100V in 0.5X TBS buffer for 3 minutes.*

***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

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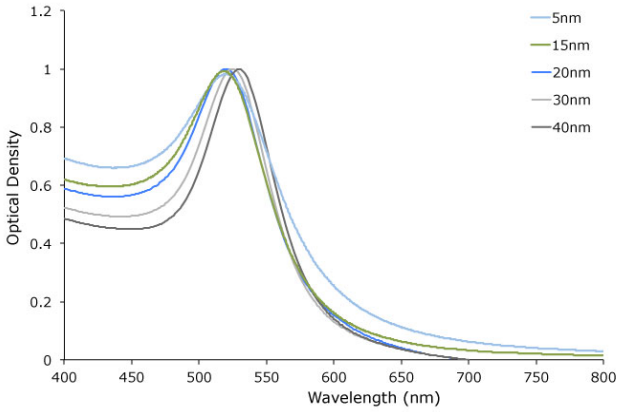
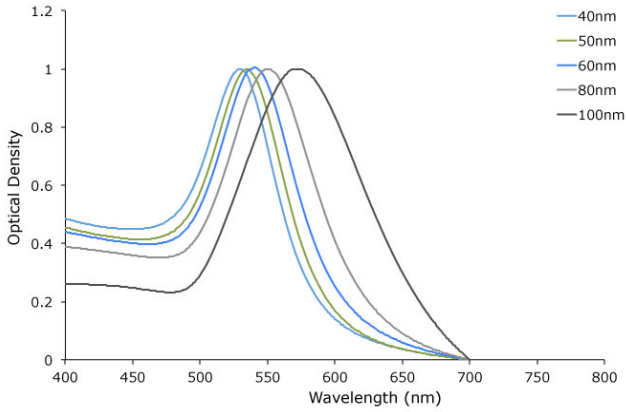
Table II. *Gold nanoparticle specifications by size. Please note that all values below are indicated at an optical density of 1 (OD/cm^{-1}) at their respective lambda max. At other optical densities the values needs to be adjusted (e.g. NPS/ml (@OD2) = 2 x NPS/ml (@OD1)).*

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





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

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