DiagNano™ Silver Nanoparticle Covalent Conjugation Kit, 100 nm

Cat. No: SCK-100

Description

DiagNano™ NHS-activated Silver Nanoparticle Conjugation Kits have been optimized for high efficiency onestep conjugations of proteins and other primary amine-containing ligands to silver nanoparticles with diameters in the size range of 10nm-100nm. The kit contains ready-to-use pre-made mixtures. No activation or manipulation of the silver nanoparticles is required prior to conjugation, which often results in poor performing conjugates. Simply mix your protein with the pre-activated NHS ester silver nanoparticles supplied in the kit. Kits are available in convenient 3 or 10 small-scale reactions formats allowing multiple to be conjugated simultaneously and ready for use in 2.5 hours or less. These kits are ideal for screening and optimization purposes prior to scale-up production. Scale up can be performed with our NHS-Activated Silver Nanoparticle Conjugation Medium kits.

Features & Benefits

- Results in covalently bound ligand and more stable conjugate.
- Fast and convenient one-step conjugation reaction with no pre-activation requirements
- Spacer between the silver nanoparticle surface and conjugated ligand minimizes effects on the tertiary protein structure, which can lead to poor performing conjugates, which a common problem seen in conjugates prepared by passive adsorption.

Applications

Ideal for development of protein silver conjugates for use in applications such as blotting, lateral flow assays, microscopy and transmission electron microscopy (TEM).

Kit Components

- NHS-Activated Silver Nanoparticles (lyophilized)
- Protein Re-suspension Buffer
- Reaction Buffer
- Quencher Solution

NHS-Activated Silver Nanoparticle Specifications

Surface functionality: NHS-ester (~ 1 NHS group/nm^2) (10kDa PEG-spacer between silver surface and NHS-group)

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Core diameter: 100nm
Concentration: OD=18 (when dissolved to a final volume of 100ul)
Particles per ml: ~7.2E+10 (when dissolved to a final volume of 100ul)
Absorbance (lambda max): 480-520nm

Storage

All components of this kit should be stored at -20°C. If stored unopened and as specified, DiagNano™ NHS-activated silver nanoparticles are stable for at least 3 months.

Factors to Consider before Conjugation

The protein/antibody or other ligand to be conjugated needs to be in a purified form, and proper care must be taken to ensure that the ligand stock is devoid of the following for proper functionality:

- No additional protein additives such as BSA
- Avoid free amino acids (e.g. glycine)
- Avoid common thiol additives such as DTT, TCEP and mercaptoethanol
- Avoid EDTA
- Avoid primary amine containing buffers or components (e.g. Tris)
- Avoid use of strong buffers that might change the pH of the conjugation reaction.

If your protein/antibody stock contains any of the above, dialyse or use a desalting column to transfer your ligand into a compatible buffer such as sodium phosphate, MOPS, MES or HEPES. If contaminating proteins such as BSA is present, the protein needs to be purified prior to conjugation.

Conjugation Procedure

A recommended starting protocol for conjugation can be found below. Note that the amount of protein added may need to be optimized for your particular protein.

1. Allow all reagents to warm to room temperature before use.
2. Using the supplied protein re-suspension buffer, dilute or dissolve your protein/antibody to the final concentration suitable for the particular silver nanoparticle size to be conjugated.
3. In a microcentrifuge tube combine your diluted protein sample with reaction buffer according to the table below.

	3 or 10 Small Kits	Medium Kits
Reaction Buffer	60 µL	600 µL
Diluted Protein Solution	48 µL	480 µL
Total Volume	108 µL	1080 µL

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4. Transfer 90 µL (900 µL for the Medium Kit) of your protein/reaction buffer mix prepared in step 3 to one of the vials containing lyophilized NHS-activated silver nanoparticles and immediately mix well by pipetting up and down.

Note: *Do not resuspend the lyophilized NHS-activated Silver nanoparticles in buffer prior to addition of protein. NHS rapidly hydrolyzes in aqueous solution and may result in loss of conjugation efficiency.*

5. Incubate the vial at room temperature for at least 2 hours.
6. Add 10 µL (100 µL for Medium Kit) of quencher solution to the vial to stop the reaction.
7. Using a microcentrifuge, centrifuge the vial for 30 minutes using the appropriate speed for the silver nanoparticle size you are using according to the table below.

Silver Nanoparticle Diameter	Centrifugation Force
10 nm	100 kDa MWC Spin Column
20 nm	17000 x g
30 nm	11000 x g
40 nm	3000 x g
50 nm	2000 x g
60 nm	900 x g
80 nm	500 x g
100 nm	300 x g

8. Discard the supernatant containing unbound protein.
9. Add 100ul (1 mL for Medium Kit) of silver conjugate storage buffer to the vial to re-suspend your conjugate.

Note: *A silver conjugate storage buffer is not supplied with the kit. Use a standard biological buffer compatible with your protein.*
A recommended storage buffer for an antibody silver conjugate is 20 mM Tris (pH 8.0), 150 mM NaCl supplemented with 1% (w/v) BSA and 0.025% Tween 20.

10. Record the UV-VIS spectra of the conjugate using a spectrophotometer, and dilute to desired optical density using silver conjugate storage buffer.
11. Store your protein conjugate at 4°C until use.

Your conjugate is now ready for use!

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Table I. *Suggested protein concentrations to be used for step 2 in the conjugation protocol above based on the silver nanoparticle size to be conjugated. Note that the concentrations in the table below are optimized for an antibody with a molecular weight of 150kDa. For proteins differing significantly in molecular weights the amounts indicated might need to be optimized for optimal performance.*

Silver Nanoparticle Diameter	Suggested Protein Concentration
10nm	3 mg/ml
20nm	1 mg/ml
30nm	1 mg/ml
40nm	0.5 mg/ml
50nm	0.5 mg/ml
60nm	0.5 mg/ml
80nm	0.5 mg/ml
100nm	0.5 mg/ml

Table II. *Silver nanoparticle specifications by size. Please note that all values below are at the optical density indicated in the table (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	Peak SPR Wavelength (nm)	NPS/ml*	Wt. Conc (mg/ml)*	Size Dispersity (+/-nm)	Particle Volume (nm3)	Surface Area (nm2)	Surface/ Volume Ratio	Particle Mass (g)	Molar Mass (g/mol)	Molar Concentration*
10	390-405	~3.6E+12	~0.02	<18%	5.24E+02	3.14E+02	0.6	5.49E-18	3.31E+06	5.98E-09
20	390-410	~4.6E+11	~0.02	<15%	4.19E+03	1.26E+03	0.3	4.39E-17	2.65E+07	7.64E-10
30	400-410	~1.4E+11	~0.02	<15%	1.41E+04	2.83E+03	0.2	1.48E-16	8.93E+07	2.32E-10
40	405-425	~5.7E+10	~0.02	<15%	3.35E+04	5.03E+03	0.15	3.52E-16	2.12E+08	9.47E-11
50	410-430	~2.9E+10	~0.02	<12%	6.54E+04	7.85E+03	0.12	6.87E-16	4.13E+08	4.28E-11
60	425-450	~1.7E+10	~0.02	<12%	1.13E+05	1.13E+04	0.1	1.19E-15	7.14E+08	2.82E-11
80	440-480	~7.1E+09	~0.02	<12%	2.68E+05	2.01E+04	0.075	2.81E-15	1.69E+09	1.18E-11
100	480-520	~3.6E+09	~0.02	<10%	5.24E+05	3.14E+04	0.06	5.49E-15	3.31E+09	5.98E-12

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