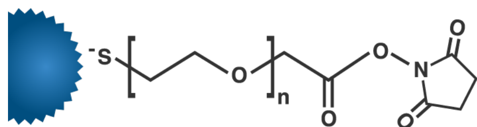
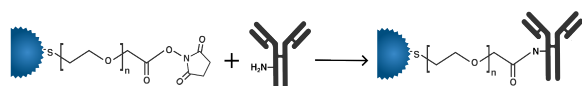
CYTODIAGNOSTICS

PRODUCT DATA SHEET

NHS-Activated Gold NanoUrchins Conjugation Kit



NHS Activated



Description

Cytodiagnostics NHS-activated Gold NanoUrchin Conjugation Kits have been optimized for high efficiency one-step conjugations of proteins and other primary amine-containing ligands to gold nanourchins with diameters in the size range of 50nm-100nm.

The kit contains ready-to-use pre-made mixtures. No activation or manipulation of the gold nanourchins is required prior to conjugation, which often results in poor performing conjugates. Simply mix your protein with the pre-activated NHS ester gold nanourchins 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 Gold NanoUrchin Conjugation MIDI 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 gold nano-urchin surface and conjugated ligand minimizes effects on the tertiary protein structure, which can lead to poor performing conjugates, which is a common problem seen in conjugates prepared by passive adsorption.

Applications

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

Kit Components

- NHS-Activated Gold NanoUrchins (lyophilized)
- Protein Re-suspension Buffer
- Reaction Buffer
- Quencher Solution

NHS-Activated Gold NanoUrchin Specifications

Gold surface: NHS-ester (spacer between gold surface and NHS-group)
Core diameter: Available with diameters from 50nm-100nm
Optical density (OD): OD=20 when the contents of each vial is dissolved to a final volume of 100ul (1ml for MIDI Kit).
Particles per ml: Core size dependant, please see table II.
Lambda max: Core size dependant, please see table II.
Supplied in ready to use lyophilized format.

Storage

All components of this kit should be stored at -20°C. If stored unopened and as specified, Cytodiagnostics NHS-activated gold nano-urchins are stable for at least 3 months.

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 (SDS) available online for proper safety and handling procedures.

Factors to Consider Prior to 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. See paragraph below for recommended buffers for optimal performance of the kit.

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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, MES, MOPS or HEPES. If contaminating proteins such as BSA is present, the protein needs to be purified prior to conjugation.

Conjugation Protocol

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 a final concentration of 0.5 mg/ml.

   Note: *For effective conjugation, the purity of the protein needs to be considered. Any other molecules containing primary amines (e.g. TRIS) or other contaminating proteins (e.g. BSA) may compete with the protein to be conjugated and hence severely reduce the conjugation efficiency and should therefore be avoided. Consider using BSA Removal Kit for Nanoparticle Conjugation (SR-08-01).*

3. In a microcentrifuge tube combine your diluted protein with reaction buffer according to the table below.

	3 or 10 Small Scale Reaction Format Kits	Midi Kits
Reaction Buffer	84µl	840µl
Diluted Protein Solution	24µl	240µl
Total Volume	108µl	1080µl

4. Transfer 90µl of your protein/reaction buffer mix prepared in step 2 to one of the vials containing lyophilized NHS-activated gold nano-urchins and immediately mix well by pipetting up and down.

   Note: *Do not resuspend the lyophilized NHS-activated gold nanourchins 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 2 hours.

6. Add 10µl (100µl for MIDI Kit) of quencher solution to the vial and incubate for 5 minutes to stop the reaction.

7. (Optional Step) - Add 10µl (100µl for Midi Kit) of 10% bovine serum albumin (BSA) to the vial and incubate for 5 minutes to block.

Gold NanoUrchin Diameter	Centrifugation Force
50nm	*2,000 x g*
60nm	*1,125 x g*
70nm	*900 x g*
80nm	*600 x g*
90nm	*500 x g*
100nm	*400 x g*

8. Using a microcentrifuge, centrifuge the vial for 30 minutes using the appropriate speed for the gold nanourhcin size you are using according to table above.

9. Discard the supernatant containing unbound protein.

10. Add 1mL of gold conjugate storage buffer to the vial to re-suspend your conjugate.

11. Repeat step 8-9 to wash away additional unbound protein.

12. Add 100µl (1mL for Midi Kit) of gold conjugate storage buffer to the vial to re-suspend your conjugate. Brief sonication might be required to fully redisperse the conjugate.

****Note:*** *A gold 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 gold conjugate is 20mM Tris (pH 8.0), 150mM NaCl supplemented with 1% (w/v) BSA and 0.025% Tween 20.

13. Obtain a UV-VIS spectra of the conjugate using a spectrophotometer and dilute to an optical density of 10 using gold conjugate storage buffer.

14. Store your protein conjugate at 4°C until use.

15. (Optional Step) - Perform a conjugation quality control test to ensure a successful conjugation reaction using our Conjugation QC Lateral Flow Dipstick Kit (Cat. # LF-018-10).

Your conjugate is now ready for use!

Purification of Nanoparticle Conjugates Using CytoColumn™

1. **IMPORTANT:** If your product or any downstream applications are sensitive to glycerine, make sure to rinse the filtration device with ddH_2O or buffer before use. Trace amounts of glycerine are present in the filtration membrane to prevent drying out.

2. Transfer your conjugated sample into the appropriate CytoColumn™ (see Page 5).

   Note I. Ensure that the molecular weight cut-off (MWCO) of the CytoColumn™ is suitable for the components being filtered out (i.e., the reactants being removed should have a lower molecular weight than the cut-off of the column). The recommended MWCO is 100 kDa for nanoparticle products.

   Note II. Do not overfill the CytoColumn™, such that there is still some space left. This will mitigate any leakage between the two column components during centrifugation.

3. Using a suitable centrifuge, centrifuge the columns according to the table below, making sure to always use a counterbalance. If there is more volume than the filter device can hold, the remainder of the sample or any wash solutions can be poured into the unit on top of the purified product and centrifuged again. Make sure to always empty contents collected at the bottom of the tube between each centrifugation.

   Table 1. *Recommended centrifugation speeds and times for different volume CytoColumn™.*

Column Size	Centrifugation Speed (x g)	Centrifugation Time
0.5 mL	10,000	10 min.
4 mL	1,700	10 min.
15 mL	1,700	10 min.

   Note. Centrifugation times will vary based on the MWCO, with smaller MWCO devices requiring longer centrifugation. If the remaining volume of purified product is more than desired, subsequent centrifugations can be done.

4. Following centrifugation, carefully collect the purified product using a micropipette. A small volume of collection buffer can be used to rinse and collect any leftover product on the membrane.

   Note: The CytoColumn™ can be re-used but ensure that the membrane does not dry out between uses. In the event of drying out, the CytoColumn™ is no longer useable.

5. The purified product is now ready for analysis and any subsequent downstream applications.

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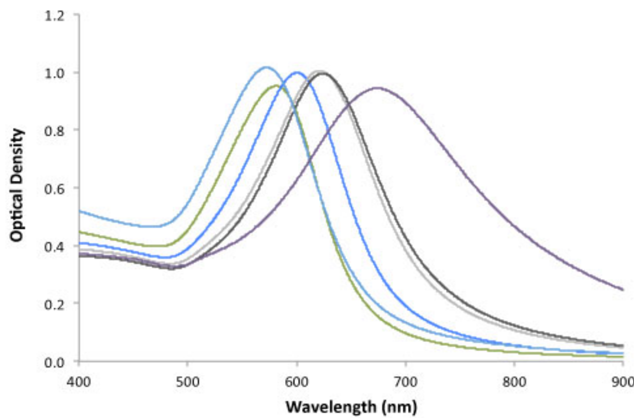
Table II. *Gold nano-urchin specifications by size. Please note that all values below are indicated at an optical density of 1 (OD/cm-1) at their respective lambda max. At other optical densities the values needs to be adjusted (e.g. NPS/ml (@OD2) = 2 x NPS/ml (@OD1)).*

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.
50	585	3.51E+10	4.45E-02	1.72E+10	<8%	6.54E+04	7.85E+03	0.12	1.27E-15	7.64E+08	5.83E-11
60	585	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	600	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	620	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	630	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	680	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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Catalog Number	Description	Sizes
GUN10K-50-X*	50nm NHS-Activated Gold NanoUrchin Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
GUN10K-60-X*	60nm NHS-Activated Gold NanoUrchin Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
GUN10K-70-X*	70nm NHS-Activated Gold NanoUrchin Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
GUN10K-80-X*	80nm NHS-Activated Gold NanoUrchin Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
GUN10K-90-X*	90nm NHS-Activated Gold NanoUrchin Conjugation Kit	3 reactions, 10 reactions, MIDI Kit
GUN10K-100-X*	100nm NHS-Activated Gold NanoUrchin Conjugation Kit	3 reactions, 10 reactions, MIDI Kit

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

Catalog Number	Description	Sizes
MWC-3-X*-Y*	MWCO Ultrafiltration Spin Columns, 3 kDa	0.5ml, 4ml, 15ml
MWC-10-X*-Y*	MWCO Ultrafiltration Spin Columns, 10 kDa	0.5ml, 4ml, 15ml
MWC-30-X*-Y*	MWCO Ultrafiltration Spin Columns, 30 kDa	0.5ml, 4ml, 15ml
MWC-100-X*-Y*	MWCO Ultrafiltration Spin Columns, 100 kDa	0.5ml, 4ml, 15ml

X* Indicates column volume, 05 for 0.5ml, 4 for 4ml, 15 for 15ml

Y* Indicates number of columns, 1 for 1 column, 5 for 5 columns. i.e. MWC-3-05-1, 3kDa cut-off, 0.5ml column pkg size of 1

Ordering Information

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