

## Silver Conjugation Kit (Covalent Conjugation) Protocol

### Kit Components

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

### 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:

1. No additional protein additives such as BSA
2. Avoid free amino acids (e.g. glycine)
3. Avoid common thiol additives such as DTT, TCEP and mercaptoethanol
4. Avoid EDTA
5. Avoid primary amine containing buffers or components (e.g. Tris)
6. 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 $\mu$ L	600 $\mu$ L
Diluted Protein Solution	48 $\mu$ L	480 $\mu$ L
Total Volume	108 $\mu$ L	1080 $\mu$ L

- Transfer 90  $\mu$ L (900  $\mu$ L 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.*

- Incubate the vial at room temperature for at least 2 hours.
- Add 10  $\mu$ L (100  $\mu$ L for Medium Kit) of quencher solution to the vial to stop the reaction.
- 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

- Discard the supernatant containing unbound protein.
- 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.*

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

## Suggested Protein Concentration

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