

Water Soluble Nanocrystal Protein Labeling

Research use only

REAGENTS

2-Mercaptoethanol	FW 78.13, >98%	Sigma M3148
EDC (1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide hydrochloride)	FW 191.7	Pierce 22980
Sulfo-NHS N-Hydroxysulfosuccinimide	FW 217.14	Pierce 24510
PIPES (1,4-Piperazinebis (Ethanesulfonic Acid) Buffer)	FW 195.24 >98.5%	Acros Organics Cat. No. AC172610250
Ethanolamine	FW 61.08 >99%	Acros Organics CAS# 141-43-5
TBS Tris Buffered Saline Powder	To yield 0.05M TBS	Sigma. T-6664
dd H₂O		
NaOH solution	1 N (Fisher certified)	Fisher SS266-1
Amicon Ultra Centrifugal Filter Tubes, 100,000 MWCO		
Dialysis membrane (optional)	Cellulose tubing or Slide-a-lyzer 100,000 MWCO	Fisher Pierce
Millipore mixed cellulose ester membrane filters (optional)	0.025µm	Fisher VSWP or VSMP
Crystalplex Carboxyl Water-soluble Nanocrystals		
Protein to be conjugated		
Water bath sonicator		Branson

REAGENT PREPARATION

1. Prepare 0.025 M PIPES buffer solution and adjust pH to 7.0 with 1N NaOH solution.
2. Prepare 0.05 M TBS using fresh dd H₂O. Adjust pH to 7.4.
3. Prepare 0.02 M EDC in deionized water (must be prepared just prior to use).
4. Prepare 0.05 M Sulfo-NHS in deionized water (must be prepared just prior to use).
5. Prepare 0.02 M Ethanolamine in deionized water.

Note

EDC powder is stored frozen -20 °C. The vial should be warmed to room temperature before opening. The material will react with moisture with subsequent loss of shelf life.

Crystalplex Nanocrystal labeling procedure

NANOCRYSTAL PREPARATION

1. Sonicate the stock solution of carboxyl nanocrystals for 30 seconds
2. Prepare a 200 µg/ml solution of nanocrystals in 0.025M PIPES buffer and vortex for 5 seconds to mix.

NANOCRYSTAL ACTIVATION – For 2 mg water soluble nanocrystals

1. Add EDC and Sulfo-NHS to the nanocluster solution in the following amounts.
10 ml nanocluster solution (200 µg/ml)
0.975 ml EDC solution (0.02M)
0.650 ml Sulfo-NHS solution (0.05M)
2. Incubate at room temperature for 10 minutes with gentle stirring or shaking. Check pH to ensure that it is between 6.7- 7.0.
3. Add 28 µL of 2-Mercaptoethanol (neat) to the activated nanocluster solution to passivate any excess EDC/Sulfo-NHS intermediate in solution.

PROTEIN ATTACHMENT

1. Prepare protein solution in 0.025 M PIPES or DI H₂O to a concentration of 1 mg/ml (typical for a 60KD protein). Concentration may vary depending on protein MW.
2. Add 500 µL of protein solution to the activated nanocrystals (2mg nanocrystals in 10 ml).
3. Incubate at room temperature for 60 minutes with gentle stirring or shaking.
4. Quench the reaction with 500 µL of 0.02 M ethanolamine. Alternatively, dialyze solution against 0.05 M TBS to quench.
5. Remove excess protein by filtering with Amicon Ultra Centrifugal Filter Tubes, 100,000 MWCO. Wash 3 times with 4 ml of dd H₂O (spin 10 min. @ 4000 rpm). After first wash, you may need to add 1 drop of 1N Sodium Hydroxide to help nanocrystals solubilize in water. Do this only if the nanocrystals do not visibly come off of the filter and tube walls.
6. Resuspend nanoclusters in dd H₂O or salt/buffer of choice. Do not exceed 160 mM total ionic strength. Shake, vortex, or sonicate to resuspend. If conjugate does not resuspend easily, add 1 drop of 1N NaOH, then readjust pH afterwards as necessary.

Note:

1-2% BSA or PEG may be added to reduce nonspecific binding and aggregation.
0.01 M sodium azide may be added as a preservative

7. Store labeled nanocrystals refrigerated at 4 °C.

REFERENCES

- 1) Hermanson, G.T., 1996, **Bioconjugate Techniques**, Elsevier Science San Diego, CA 785 pp.
- 2) Graberek, Z. and Gergely, J., 1990, *Anal. Biochem* 185:131-135.