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Protocol to link 250µl CPNs to proteins (including antibodies or streptavidin).

Materials:

CPN™550 (Yellow) 0.1mg/ml	Stream Bio	# 5500B02
HEPES solution, 1M, pH 7.0-7.6	Sigma	#H0887-20ML
Polyethylene glycol 8000 (PEG)	Sigma	#89510-250G-F
N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride] (EDC)	Sigma	#E7750-100MG
Bovine Serum Albumin (BSA), heat shock fraction	Sigma	# A7906-10G
TWEEN(R) 20 polyoxyethylenesorbitan mono	Sigma	# P1379-25ML
Distilled water		
1.5ml tubes		
Magnetic rack or neodymium magnet		
Goat Anti-Mouse IgG H&L 1.0MG (Lot No: GR296300)	Abcam	# ab6708

(Protein used in linkage must be free of sodium azide and bulking proteins (e.g. BSA). If these are present, remove them using the BSA Removal Kit, Abcam # ab173231 following manufacturer's instructions and resuspending the protein at 1.0mg/ml).

Methods:

- 1) Use 250µl CPN550 (0.1mg/ml) in 1.5ml tube.
- 2) Add 5µl HEPES pH7.4 (1M) to the diluted CPN.
- 3) Make up ~1ml PEG 5% w/v (50mg/ml) in 1.5ml tube:

Empty tube	1.1061g	
+ PEG	1.1338g	
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PEG mass	0.0277g	add 554µl to make 50mg/ml (mix well)

- 4) Add 5µl PEG 5% to CPN mixture.

5) Make up protein to be linked e.g. antibody or streptavidin (1mg/ml) by adding 100µl distilled water to 0.1mg protein.

6) Add 15µl protein e.g. anti-mouse IgG (1mg/ml) to CPN mixture.

7) Make up ~1ml EDC (5mg/ml) in 1.5ml tube immediately before use:

Empty tube	1.1079g	
+ EDC	1.1116g	

EDC mass	0.0037g	add 740µl to make 5mg/ml (mix well)

8) Add 5µl EDC to CPN mixture.

9) Incubate at room temperature for 4 hours with gentle mixing (6 rev/min on rotary mixer).

10) Separate CPN mixture using magnetic rack overnight at 4°C.

11) Remove supernatant and resuspend in 100µl sterile Resuspension Buffer.
(HEPES (20mM), BSA 0.1%, Tween-20 0.02%, pH7.4)

12) Measure sample fluorescence and compare to reference sample 0.1mg/ml and adjust to 0.1mg/ml CPN with sterile Resuspension Buffer.

Disclaimer:

This protocol and, the CPNs™, associated reagents / materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining Support Search FAQ's <https://www.streambio.co.uk/faq/> or submit a question directly to Technical Support techsupport@streambio.co.uk. Other documents such as product user documents,

SDSs, and application notes, formulations, handbooks, and other product support documents are also available from the website www.streambio.co.uk

SDS Safety Data Sheets (SDSs) are available at <https://www.streambio.co.uk/resources-downloads/>

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