CPNs™	Ex/Em maxima (nm)	Fluorescent colour	Spectrally comparable* fluorophores	Commonly used filter sets (Ex/Em)
CPN™ 1000	750 / 1000	IR-II		
CPN™ 840	630 / 840	IR-I		
CPN™ 830	610 / 830	IR-I		
CPN™ 820	640 / 820	IR-I		
CPN™ 770	610 / 770	IR-I		
CPN™ 680	400 / 680	Red		
CPN™ 660	540 / 660	Red		
CPN™ 610	480 / 610	Orange		
CPN™ 580	488 / 580	Orange		
CPN™ 550	470 / 550	Yellow	FITC, Alexa Fluor®	475/70 BP
			488, GFP, YFP	530/86 BP
CPN™ 530	455 / 530	Green		
CPN™ 510	400 / 510	Green		
CPN™ 510	455 / 510	Green	FITC, Alexa Fluor®488,	475/70 BP
			Dylight 488,GFP, YFP	530/86 BP
CPN™ 475	390 / 475	Blue	AMCA, eBFP, DAPI,	377/60 BP
			Hoechst 33342,	447/60 BP
			Hoechst 33258	
CPN™ 435	390 / 435	Indigo	AMCA, eBFP, DAPI,	377/60 BP
			Hoechst 33342,	447/60 BP
			Hoechst 33258, Alexa	
			Fluor® 405	
CPN™ 420	390 / 420	Violet	AMCA, eBFP, DAPI,	377/60 BP
			Hoechst 33342,	447/60 BP
			Hoechst 33258, Alexa	
			Fluor® 405	

LINKBRIGHTTM Conjugation Kits

are available for IgG antibody, oligonucleotide and protein linkage via Amine & Thiol in the above CPN wavelengths.

Purchaser Notification

These high-quality reagents and 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 for each product available, other regulatory considerations may apply.

Obtaining Support Search FAQ's at www.streambio.co,uk/FAQs or submit a question directly to

Technical Support techsupport@streambio.co.uk

Safety Data Sheets (SDSs) are available at www.streambio.co.uk/resources-

information for each product. Certificates of Analysis are available on request

downloads/

Certificate of Analysis The Certificate of Analysis provides detailed quality control and product qualification

For Research Use Only. Not for use in diagnostic procedures.

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LINK**BRIGHT** TM

CPN: Amine IgG Antibody Conjugation Kit

Manual and protocol

30min reaction time, 5 easy steps

This product is for research use only and is not intended for diagnostic use.

Materials supplied and storage

Vial / cap colour	Item (colour coded)	Quantity		Storage
1. White*	(150µI) LINK BRIGHT ™- Amine	1 vial	3 vials	-20°C
2. Blue	IgG antibody purification reagent§	1 vial	1 vial	4°C
3. Red	Solution R	1 vial	1 vial	-20°C
4. Green	Solution SG	1 vial	1 vial	-20°C
5. Yellow	Solution Z	1 vial	1 vial	-20°C

When stored as directed, the kit components are stable for at least 6 months. 1x vial of CPN LINKBRIGHT™ (150µI) is optimised for ~10µg of antibody. The kits are not suitable for purifying labeling IgM.

*CPNsTM are available in a range of emission wavelengths (420 nm to 1000 nm in the IR see table or visit www.streambio.co.uk/products/
§lgG antibody purification reagent can be purchased separately from Stream Bio cat. no. IGP0K11 - vial #2 – blue cap)

Procedure Overview

(Hands-on) - Add antibody to a vial of LINK**BRIGHT**™

Reaction time 30 min

Stop reaction

(Hands-on) - Add Solution SG for 5 min

Before you Start

IMPORTANT: The purified IgG must be in a buffer free of ammonium ions, primary amines, or sodium azide preservatives, as they will disrupt the linkage reaction with the CPNs™ If the IgG is in, or has been lyophilized from an unsuitable buffer (e.g. Tris or glycine) or purified with ammonium sulphate, the buffer needs to be replaced with HEPES. IgG antibodies can be purified and resuspended using the procedure below. To prepare other reagents for linkage use standard methods for those material, e.g. microdialysis or column separation. Impure antibodies, antibodies stabilized with bovine serum albumin (BSA) / gelatin or contain antimicrobials such as sodium azide will not label well and should be purified using the IgG antibody purification procedure described.

Biomolecule Conjugation Procedure

IgG antibody purification* (optional)

- 1. For every 10µl of IgG antibody (1mg/ml), add 8µl of IgG Antibody Purification reagent (vial #2, blue cap)
- 2. Incubate at room temperature for 5 min
- 3. Centrifuge at 13,000xg for 5 min, discard supernatant
- 4. Resuspend antibody in 10µl of Solution R (vial #3, red cap)

* NB: Antibody used in linkage must be free of sodium azide and bulking proteins (e.g. BSA) etc. If these are present, the reaction will not work. Remove them by following the IgG antibody purification procedure and resuspend in the provided solution R at 1.0mg/ml.

Note: LINKBRIGHT™ Amine Antibody Conjugation Kit is optimised for conjugation of IgG antibodies to the amine group, for other biomolecules please either use an alternative kit, consult our guide, or contact technical support. LINKBRIGHT™ Oligonucleotide Conjugation Kits are also available

CPN Conjugation

- 1. Add 10 μl (10 μg)§ of antibody to a vial of LINKBRIGHTTM CPN Amine
- 2. Incubate at room temperature for 30 min
- 3. Add 6 µl Solution SG (vial #4, green cap)
- 4. Incubate at room temperature for 5 min
- 5. Add 6 μl of Solution Z**(vial #5, yellow cap)

(Optional) A magnetic separation[‡] method can be chosen to purify the conjugates when required

§optimal antibody to CPN ratio to be determined by end user.

**Adding Solution Z (vial #5, yellow cap) is optional. Solution Z contains protein stabiliser for long term storage.

[‡]CPNs[™] can be attracted to magnets allowing the purifying and separating of the CPNs[™] from unlinked reagents.