

| CPNs™ | Ex/Em maxima (nm) | Fluorescent colour | Spectrally comparable* fluorophores | Commonly used filter sets (Ex/Em) |
|-----------|-------------------|--------------------|--|-----------------------------------|
| CPN™ 1130 | 750 / 1130 | NIR-II | | |
| CPN™ 900 | 650 / 900 | NIR-I | | |
| CPN™ 680 | 400 / 680 | Red | | |
| CPN™ 610 | 480 / 610 | Orange | | |
| CPN™ 550 | 470 / 550 | Yellow | FITC, Alexa Fluor® 488, GFP, YFP | 475/70 BP 530/86 BP |
| CPN™ 510 | 400 / 510 | Green B | | |
| CPN™ 510 | 450 / 510 | Green | FITC, Alexa Fluor®488, Dylight 488,GFP, YFP | 475/70 BP 530/86 BP |
| CPN™ 475 | 390 / 475 | Blue | AMCA, eBFP, DAPI, Hoechst 33342, Hoechst 33258 | 377/60 BP 447/60 BP |
| CPN™ 435 | 390 / 435 | Indigo | AMCA, eBFP, DAPI, Hoechst 33342, Hoechst 33258, Alexa Fluor® 405 | 377/60 BP 447/60 BP |
| CPN™ 420 | 390 / 420 | Violet | AMCA, eBFP, DAPI, Hoechst 33342, Hoechst 33258, Alexa Fluor® 405 | 377/60 BP 447/60 BP |



LINKBRIGHT™

CPN: Thiol IgG Antibody Conjugation Kit

Manual and protocol

60min reaction time, 5 easy steps

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

LINKBRIGHT™ 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

SDS

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

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on request

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

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Stream Bio Ltd and/or its affiliate(s) warrant their products as set forth in the Stream Bio' General Terms and Conditions of Sale found on Stream Bio's' website at www.streambio.co.uk/termsandconditions If you have any questions, please contact Stream Bio Ltd at info@streambio.co.uk or techsupport@streambio.co.uk

Materials supplied and storage

| Cap colour | Item (colour coded) | Quantity | | | Storage |
|------------|--|----------|---------|----------|---------|
| 1. Grey* | (150µl) LINKBRIGHT™ Thiol | 1 vial | 3 vials | 10 vials | -20°C |
| 2. Blue | IgG antibody purification reagent [§] (200µl) | 1 vial | 1 vial | 1 vial | 4°C |
| 3. Purple | Solution RA (200µl) | 1 vial | 1 vial | 1 vial | -20°C |
| 4. Orange | Solution SC (200µl) | 1 vial | 1 vial | 1 vial | -20°C |
| 5. Yellow | Solution Z (200µl) | 1 vial | 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™ (150 µL) is optimised for ~10 µg of antibody.

The kits are not suitable for purifying labeling IgM.

*CPNs™ are available in a range of emission wavelengths (420 nm to 1130 nm in the IR see table or visit www.streambio.co.uk/products/

[§]IgG antibody purification reagent can be purchased separately from Stream Bio cat. no. **IGP0K11** IgG antibody purification reagent - vial #2 – blue cap)

Procedure Overview

(Hands-on) – Reduce thiol groups on antibody

Reaction time 30 min

(Hands-on) - Add antibody to a vial of LINKBRIGHT™

Reaction time 30 min

Stop reaction

(Hands-on) - Add Solution SC 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 10 µl of IgG antibody (1 mg/ml), add 8 µl 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 10 µl of Solution RA (vial #4, purple cap)
5. Incubate at RT for 30 min and followed by CPN conjugation procedure

* 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 RA at 1 mg/ml.

Note: LINKBRIGHT™ Thiol Antibody Conjugation Kit is optimised for conjugation of IgG antibodies to the thiol 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 LINKBRIGHT™ CPN - Thiol
2. Incubate at room temperature for 30 min
3. Add 6 µl Solution SC (vial #4, orange 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.