COLUMBIA BIOSCIENCES

SURELIGHT® ALLOPHYCOCYANIN (SL-APC) CONJUGATION KIT

Complete reagent kit for small-scale conjugation of up to 1mg of antibody

Cat. #: D3-4500

6440 Dobbin Road, Suite D
Columbia, MD 21045
Phone (443) 430-0403 Toll Free: (866) 568-5772
Fax (443) 430-0407
www.columbiabiosciences.com email: info@columbiabiosciences.com

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KIT CONTENTS

Item	Qty
Activated SL-APC; [enough for 1 mg of IgG] (amber tube)	3 mg
Dithiothreitol (DTT) Stock Solution; [1 M] (yellow cap)	0.1 ml
Blue Dextran Stock Solution; [50 mg/ml, 25x] (purple cap)	0.1 ml
N-ethylmaleimide (NEM) Stock Solution; [10 mg/ml] (green cap)	0.1 ml
Exchange Buffer	50 ml
Storage Buffer	50 ml
dd Water	50 ml
Desalting Column A	1
Desalting Column B	1
Desalting Column Collection Tubes	2
Ultrafilter® plus Filtrate Collection Tube	2
Bio-Spin® Column plus Wash Tube and Collection Tube	2

STORAGE CONDITIONS

- Kits are shipped with a cold pack for next day delivery, and are warranted for six months from date of shipment if stored at 4°C.
- SL-APC solutions, once reconstituted, and Desalting/Spin Columns should not be frozen.
- For optimal performance, DTT and NEM Stock Solutions should be stored at -20°C (frozen) if this kit will not be used within 30 days.
- The final conjugate should be protected from light and stored at 4°C. Once reconstituted, DO NOT FREEZE.

INTRODUCTION

Activated SureLight® Allophycocyanin (SL-APC) is a highly fluorescent phycobiliprotein which has been chemically activated for easy conjugation to reduced immunoglobulins or other sulfhydryl-containing proteins. The SL-APC has been activated with SMCC (succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate) under conditions that result in modification of only a few lysine residues on each SL-APC molecule. The reactive group introduced by activation is maleimide, which readily reacts with reduced cysteines and other sulfhydryl residues under mild conditions of temperature and pH.

The fluorescent properties of Activated SL-APC are stable at 4°C for several months, both before and after conjugation. Although the number of maleimide groups on Activated SL-APC will decline slowly over time, its performance in standard antibody conjugation assays is not substantially reduced after several months at 4°C in the provided buffer. The conjugation protocol offers the following advantages:

- a well-defined procedure
- no interference with the antibody binding site
- good conjugates over a range of antibody concentrations

The protocol is written to conjugate 1 mg of IgG using Desalting Columns A and B. If antibody supplies are limited or cost-prohibitive, Ultrafilters and Spin Columns are included to enable smaller conjugations that approximate the same reaction conditions. Suggestions for use of Desalting Columns, Ultrafilters and Spin Columns are included in the TIPS section in this booklet.

The conjugation protocol is divided into four steps:

A. Antibody Preparation - Purified antibody at 1.5–2.5 mg/ml should be formulated in a buffer which will not interfere with DTT reduction. The exchange buffer provided in the kit works well for antibody preparation.

B. Antibody Reduction - Antibody is treated with DTT to expose free sulfhydryls, and then excess DTT is removed by desalting chromatography. Two alternative desalting procedures are provided; the choice depends on the volume of reduced antibody.

C. Covalent Conjugation - Activated SL-APC is covalently coupled to the reduced antibody through reaction of the maleimide groups with the free sulfhydryl groups on the antibody. Any remaining free sulfhydryl groups are then covalently blocked by treatment with NEM.
   A significant molar excess of SL-APC is added to ensure that the reaction works as reliably as possible without requiring the customer to optimize the reaction for each new antibody to be conjugated.

D. Conjugate Finishing - The final conjugate is exchanged into an appropriate storage buffer by desalting, which also serves to remove excess NEM. Two alternative desalting procedures are described; the choice depends on the available volume and concentration, as well as acceptable stability and yield requirements.

These protocols do not include a purification step for removal of the unreacted SL-APC. For immunocytometric staining of cell surface antigens, for instance, it is often unnecessary to perform further purification. However, for staining of intracellular antigens in fixed cells, purification is sometimes required.

Kit Flexibility

The Desalting Columns (A and B) and Spin Columns provide alternate ways to use the kit. This redundancy allows the user to maximize the utility of the kit by using these components to best suit their needs:

1. Perform a single 1 mg antibody conjugation using Desalting Columns A and B.
2. Perform a small-scale conjugation using the two Spin Columns in place of Desalting Columns A and B. Then the remainder of the kit can be used to scale up the conjugation, or be used for conjugation of a second antibody.
3. Perform two small-scale conjugations of different antibodies using the two Spin Columns to desalt the reduced antibodies. The two Ultrafilters can be used to concentrate them (if needed). These conjugates can then be used as-is (if the NEM and DMSO in the reaction don't interfere with use of the conjugate), or can be dialyzed for buffer exchange. As with Option 2 above, this leaves the rest of the kit reagents and the Desalting Columns (A and B) available for a larger-scale conjugation with one of the two tested antibodies or a third antibody.
4. Perform three medium-scale conjugations (0.25-0.5 mg) with different antibodies: the two Spin Columns and Desalting Column A are used to desalt each reduced antibody. Then Desalting Column B can be used for buffer exchange of one conjugate, while the other two conjugates could be used as-is (if the NEM and DMSO in the reaction don't interfere with use of the conjugate), or be dialyzed for buffer exchange.

Activated SL-APC can be purchased separately for more or larger conjugations; excess quantities of the DTT, Blue Dextran and NEM solutions have been provided in the kit. Furthermore, additional flexibility can be gained by purchasing additional desalting columns, spin columns or ultrafilters (see Appendix D, Source of Materials).

The Final Conjugate

The protocol as written tends to produce high molecular weight conjugates with multiple molecules each of antibody and SL-APC. With some antibodies, there may be little difference between the conjugates produced at low and high ratios of SL-APC:IgG, but with others a 1:1 or even 2:1 molar ratio can result in some or all of the conjugate precipitating. The high ratio of SL-APC to antibody in the protocol minimizes this tendency. When scaling up conjugations, it is important to maintain similar reaction conditions. The Spin Column Procedure produces results equivalent to the Desalting Column Procedure if molar ratios and component concentrations are the same.

Purifying the Finished Conjugate

Conjugation Kits are designed for easy conjugation of antibodies (Abs) and other proteins to phycobiliproteins (PBs) and their tandems. Conjugates produced with these kits contain unincorporated PB (since PB is supplied in excess), and may also contain small amounts of unincorporated Ab.

In many applications the presence of unincorporated reactants does not compromise conjugate performance. However, further purification may be necessary to increase sensitivity or precision, or to evaluate or compare different conjugate lots. The disadvantages of purification should be considered before choosing to add this step, as losses of over 50% are possible when processing small quantities.

OVERVIEW OF PROCEDURE

Amount of antibody available to conjugate:

> 50 µg	between 25 µg and 50 µg	< 25 µg
follow desalting column procedure on page 8	Follow either desalting column or spin column procedure depending on volume of antibody	follow spin column procedure on page 11

Steps of protocol, regardless of procedure chosen:

A. Antibody preparation
B. Antibody reduction
C. Covalent conjugation
D. Conjugate finishing

NOTE: Steps such as dialysis, concentration by ultrafiltration, or buffer exchange using desalting or spin columns, result in antibody losses. When starting with very small amounts, these losses can be substantial and should be taken into consideration when planning your conjugate procedure.

Reaction Size

As little as 25 µg of antibody may be conjugated using this kit. However, some material will be lost when desalting the reduced antibody. This results in a reduced conjugate yield and may affect the conjugate size distribution; these effects are most pronounced for the smallest reactions. In general, we recommend a minimum reaction size of 100 µg, if possible.

Antibody

Purified IgG solutions at 1.5-2.5 mg/ml (2 mg/ml or higher for best results), free of BSA or other proteins.

Most buffers are acceptable (MES, phosphate and Tris have been used successfully with or without NaCl), provided they are between pH 6 and 8 and do not contain oxidizing agents or other compounds which might interfere with antibody reduction.

NOTE: If necessary, dialyze the antibody against phosphate-EDTA buffer (see Dialysis Buffer, Appendix B). We recommend concentration of the antibody if less than 2 mg/ml. Dilute antibody solutions may be concentrated using the Ultrafilter, provided with the kit (see Use of Ultrafilters to Concentrate Antibody Solutions in the TIPS section).

PROTOCOL

NOTE: Please read the entire procedure as well as the TIPS section before beginning.

NOTE: Before beginning procedure, <u>reconstitute the activated SL-APC with 385 µl of dd water</u> (provided in kit). Mix well by vortexing then centrifuge the tube before use. Once reconstituted, DO NOT FREEZE.

<u>Supplies Needed for Antibody Reduction, Desalting, and Conjugation</u>

NOTE: Collect the following supplies before starting the procedure.

<u>User supplies:</u>

Antibody solution
Rocker or rotator platform
Various size disposable pipets
Rack or ring stand capable of holding desalting or spin columns
Racks capable of holding eluate tubes or fraction collection tubes
Rack capable of holding microcentrifuge tubes
Various size pipettors
Microcentrifuge tubes
Ultracentrifuge (capable of spinning microcentrifuge tubes at 10,000 x g)
Clinical centrifuge (capable of spinning tubes at 1,000 x g)
SL-APC custom conjugation kit
Plate reader
Slide-a-lyzers or dialysis tubing (if needed to prepare antibody)

A. <u>Antibody Preparation</u>

For either conjugation protocol:

1) Dialyze antibody into appropriate buffer if necessary.

2) Once dialysis is complete, put antibody solution into a clean microcentrifuge tube and proceed to either the Desalting Column procedure or the Spin Column Procedure.

Antibody must be in an appropriate buffer for the conjugation procedure to work. In order to make sure it is in the proper buffer it may be dialyzed into buffer. The dialysis buffer or exchange buffer in Appendix B work well for this purpose.

For Dialysis, slide-a-lyzers (Pierce) or dialysis tubing may be used for the dialysis. Once antibody is prepared, proceed to either the Desalting Column Procedure or the Spin Column Procedure below.

Desalting Column Procedure

NOTE: See Use of Desalting Columns in the TIPS section.

B. Antibody Reduction

NOTE: Be careful to return caps to the proper vials.

Antibody Solution (from above)
DTT Stock Solution (supplied with kit, yellow capped tube)
Blue Dextran Stock Solution (supplied with kit, purple capped tube)
Exchange Buffer (supplied with kit)
Desalting Column A (supplied with kit)

1) Add 20 µl of DTT Stock Solution per ml of antibody solution to the tube containing the antibody solution and mix. *Example*: add 5 µl of DTT Stock Solution to 250 µl of IgG solution.

   The DTT reduction should be done in a small tube, such as a microcentrifuge tube, and capped to minimize oxygenation. Use multiple microcentrifuge tubes if the volume of the antibody solution is larger than 1.4 mL.

2) Let stand at room temperature (~ 22^{o}C) for 30 minutes without additional mixing or agitation.

3) Add 40 µl of Blue Dextran Stock Solution per ml of reduced IgG solution and mix. In the example given above (250 µl of IgG), add 10 µl of Blue Dextran Stock Solution. The Blue Dextran will elute with the antibody but will not affect the function of the antibody. It merely allows you to visualize when the antibody is coming off the column.

4) Affix Desalting Column **A** to a ring stand or similar support and remove the cap from the top of the column. Place a receptacle below the column before removing the bottom cap and allow the buffer to drain completely. Load reduced IgG/blue dextran solution unto column.

See figure 1 below

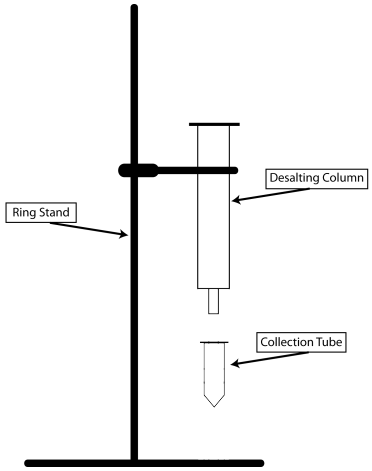

Figure 1

5) Add 25 mL of Exchange Buffer to Desalting Column A. Allow the Exchange Buffer to flow through the column until the Blue Dextran approaches the bottom of the Desalting Column A.

The Blue Dextran should migrate as a tight band through the column (see figure 2)

6) Use the opaque collection tube to collect the eluate of Desalting Column A just **before** the Blue Dextran band begins to drip from the column. Collect a total volume about 0.5 ml larger than the volume of the DTT-Blue Dextran-antibody solution initially loaded.

Approximately 90% of the Blue Dextran should have eluted from the column when you stop collecting drops. If necessary, the bottom of the column may be re-capped to stop the flow of Desalting Column A. Do not re-cap the top of the column until procedure is complete.

7) Once the antibody is eluted from the column, it may be susceptible to oxidation of sulfhydryl bonds. The time between Desalting and Conjugation steps should be minimized.

8) Proceed to Covalent Conjugation as soon as possible because the IgG sulfhydryls will re-oxidize.

C. Covalent Conjugation

Activated SL-APC (supplied in kit, amber tube)
NEM Stock Solution (supplied in kit, green capped tube)

1) Spin Activated SL-APC tube quickly in microcentrifuge before beginning to make sure all the liquid is in the bottom of the tube.

2) Add 3 mg (300 µl) of Activated SL-APC per mg of IgG to the collection tube.

Assume 100% of the antibody was recovered from Desalting Column A

3) Wrap the reaction tube in aluminum foil to protect the reaction from light and incubate for 60 minutes at room temperature (~22^{o}C) on a rocker platform or other slow mixer.

4) Meanwhile, thaw the NEM Stock Solution by warming to room temperature before use.

5) Block the unreacted free sulfhydryls on the IgG by adding 34 µg (3.4 µl) of NEM Stock Solution per mg IgG to the mixture in the collection tube and mix briefly.

6) Wrap and let stand for 20 minutes at room temperature (~22^{o}C).

Do not agitate the solution during this incubation. If time is limited after this 20 minute incubation, the reaction may be stored overnight at 4°C. Otherwise, proceed directly to Conjugate Finishing (step 17) to desalt the Antibody SL-APC Conjugate. If the conjugate is to be purified to remove unreacted proteins, skip Conjugate Finishing and go directly to the purification procedure.

D. Conjugate Finishing

Storage Buffer (supplied with kit)
Desalting Column B (supplied with kit)

1) Affix Desalting Column **B** to a ring stand or similar support and remove the cap from the top of the column. Place a receptacle below the column before removing the bottom cap and allow the buffer to drain completely.

2) Centrifuge the Antibody SL-APC Conjugate at ~10,000 *g* for 30 seconds

A pellet of insoluble conjugate aggregates may form after centrifuging. These aggregates need to be removed to prevent them clogging the column frit

3) Remove the Antibody SL-APC Conjugate supernatant and apply it to the column. Discard the pellet.

4) Apply the Antibody SL-APC Conjugate supernatant to Desalting Column **B**. Let the entire solution flow through Desalting Column **B**.

No more than 2.0 mL of Conjugated-Antibody supernatant should be applied per column.

5) Add 25 mL of Storage Buffer to Desalting Column **B**. Allow the Storage Buffer to flow through the column until the Blue Dextran approaches the bottom of Desalting Column **B**.

The blue color of the Antibody SL-APC Conjugate should migrate as a tight band through the column (see figure 2 below).

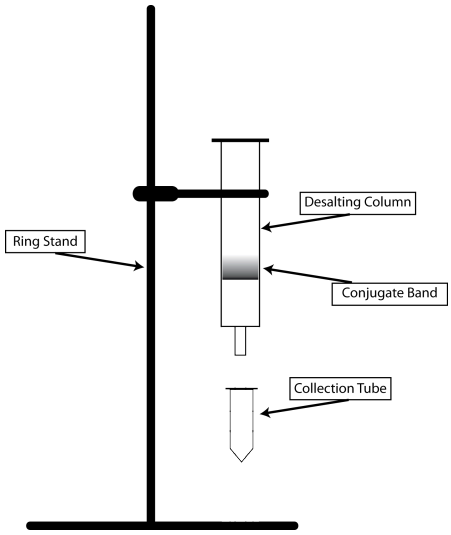

Figure 2

6) Use another opaque collection tube to collect the eluent of Desalting Column **B** just **before** the Antibody SL-APC Conjugate band begins to drip from the column. Stop collecting when nearly all of the blue color of the Antibody SL-APC Conjugate has cleared from the column.

If necessary, the bottom of the column may be re-capped to stop the flow of Desalting Column B. Do not re-cap the top of the column until procedure is completed.

7) Store the desalted Antibody SL-APC Conjugate at 4°C protected from light; DO NOT FREEZE

NOTE: The conjugate can be exchanged instead into another appropriate buffer using Column B, provided that the column is first equilibrated by passing 25–40 ml of the desired buffer through the column.

NOTE: As an alternative to using the Desalting Column, the conjugate can be exchanged into an appropriate storage buffer by dialysis. This may be useful in cases where it is advantageous to avoid further dilution.

Spin Column Procedure

NOTE: See Use of Spin Columns in the TIPS section.

B. Antibody Reduction

NOTE: Be careful to return caps to the proper vials.

Antibody Solution
DTT Stock Solution (supplied with kit, yellow capped tube)
Exchange Buffer (supplied with kit)
Spin Column (supplied with kit)

1) Start with 50 µl of antibody solution. If volume is lower, add buffer to a final volume of 50–60 µl.

2) Add 1 µl of DTT Stock Solution.

The DTT reduction should be done in a small tube, such as a microcentrifuge tube, and capped to minimize oxygenation.

3) Let stand at room temperature for 30 minutes without additional mixing.

4) While DTT reduction is incubating, equilibrate the spin column with Exchange Buffer as follows:

   a. *Snap off the tip, remove the cap, and place the tip of the column into the capless 2-ml Wash Tube.*
   b. *Swirl column gently or flick with a fingertip to set the bed evenly in the column.*
   c. *Allow the excess packing buffer to drain by gravity to the top of the gel bed; discard buffer.*
   d. *If column does not begin to flow, push the cap back onto column and remove again, this should break the surface tension. Alternatively, centrifuge for a short time.*
   e. *Fill column (~1.5 ml) with appropriate buffer.*
   f. *Centrifuge the assembly for 4 minutes in a clinical or other centrifuge with a swinging bucket rotor capable of generating a force of 1000 x g.*
   g. *Discard buffer from the Wash Tube.*
   h. *Add buffer, centrifuge and discard again.*
   i. *Without adding additional buffer, spin the column for 4 minutes to ensure complete removal of extraneous buffer. Little or no additional buffer (<50 µl) should drain into the collection tube.*

5) Place the tip of Spin Column into the Collection Tube (standard 1.5 ml Eppendorf tube supplied with Spin Column). Carefully apply 50–60 µl of reduced antibody directly to the center of the column.

Column should already be equilibrated as described above.

6) Centrifuge for 4 minutes at 1000 x g. The eluent volume should be about 80–100% of the volume of sample loaded. A clinical-type centrifuge with a swinging bucket rotor is ideal for this step.

7) Proceed to Covalent Conjugation as soon as possible because the IgG sulfhydryls will re-oxidize.

C. Covalent Conjugation

Activated SL-APC (supplied in kit, amber tube)
NEM Stock Solution (supplied in kit, green capped tube)

1) Spin Activated SL-APC tube quickly in microcentrifuge before beginning to make sure all the liquid is in the bottom of the tube.

2) Add 3 mg (300 µl) of Activated SL-APC per mg of IgG.

Assume 100% of the antibody was recovered from the Spin Column

3) Wrap the reaction tube in aluminum foil to protect the reaction from light and incubate for 60 minutes at room temperature (~22^{o}C) on a rocker platform or other slow mixer.

4) Meanwhile, thaw the NEM Stock Solution by warming to room temperature before use

5) Block the unreacted free sulfhydryls on the IgG by adding 34 µg (3.4 µl) of NEM Stock Solution per mg IgG

6) Wrap and incubate for 20 minutes at room temperature (~22^{o}C).

Do not agitate the solution during this incubation. If time is limited after this 20 minute incubation, the reaction may be stored overnight at 4°C. Otherwise, proceed directly to Conjugate Finishing (step 14) to desalt the Antibody SL-APC Conjugate. If the conjugate is to be purified to remove unreacted proteins, skip Conjugate Finishing and go directly to the purification procedure.

D. Conjugate Finishing

Storage Buffer (supplied with kit)
Spin Column (supplied with kit)
Ultrafilter (supplied with kit, optional)

1) Start with up to 100 µl of antibody solution. If volume is <50:µl, add buffer to a final volume of 50–100 µl

2) Centrifuge the Antibody SL-APC Conjugate at ~10,000 *g* for 30 seconds

A pellet of insoluble conjugate aggregates may form after centrifuging. These aggregates need to be removed to prevent them clogging the column frit

3) Remove the supernatant and apply it to the column. Discard the pellet.

4) Place the tip of Spin Column (equilibrated with Storage Buffer and drained as described on p. 14) into the Collection Tube (standard 1.5 ml Eppendorf tube supplied with Spin Column).

5) Carefully apply up to 100 µl of conjugate directly to the center of the column.

6) Centrifuge for 4 minutes at 1000 x g. The eluent volume should be 80–100% of the volume of sample loaded. A clinical-type centrifuge with a swinging bucket rotor is ideal for this step.

7) Store the final conjugate at 4°C protected from light; DO NOT FREEZE

TIPS

NOTE: It may be useful to spin SL-APC-IgG conjugates prior to use in staining (e.g., at 10,000 x g in a microcentrifuge at 4°C), especially if background seems to be a problem.

A. Use of Ultrafilters to Concentrate Antibody Solutions

NOTE: If unfamiliar with the use of these devices, we recommend the user first test the centrifuge setup by spinning the empty cartridge before adding the antibody solution.

NOTE: Molecular weight cutoff of Ultrafilter membrane is 30 kD.

1. Pour or pipette up to 0.5 ml antibody solution into the Ultrafilter. Close the cap tightly.
2. Place the tip of the Ultrafilter into the Filtrate Collection Tube and put the assembly into a centrifuge with a rotor that accommodates 2.2 ml centrifuge tubes.
3. Spin up to 10,000 x g, checking periodically until the desired final retained volume (50–60 µl) is obtained.
4. Transfer the concentrated antibody solution into a microcentrifuge tube.
5. The Ultrafilter should not be reused.
6. Additional units may be purchased directly from Millipore Corporation (#UFV5BTK00).

B. Use of Blue Dextran

Blue Dextran may be added to the sample during antibody reduction as a marker; only colored fractions need to be collected from the Desalting Column A (Blue Dextran is not necessary with the Spin Column Procedure). This makes collection of reduced antibody easier as the need to monitor absorbance of the eluted fractions is avoided. Those fractions containing the peak of Blue Dextran can be pooled.

Blue Dextran should not be added if its presence in the final conjugate will compromise performance. In general, Blue Dextran does not interfere with the use of conjugates in FACS applications. If Blue Dextran is not added, fractions will need to be collected from Desalting Column A. It is recommended that fractions be 250 µl to 500 µl in size.

C. Use of Desalting Columns

The Desalting Columns are pre-equilibrated with Exchange Buffer (Column A) and Storage Buffer (Column B). When using them, allow buffer or sample to drain into the column before proceeding to the next step. The presence of the upper frit will ensure the column will not run dry, even if unattended.

1. Care should be taken when loading samples to minimize dilution.
2. After allowing the sample to enter the gel matrix, wash it into the matrix with a small volume (*i.e.* 0.25 ml) of buffer. The upper reservoir can then be filled with a larger volume of buffer and eluting fractions collected.
3. Columns may be recycled by washing with at least 25 ml of appropriate buffer.
4. Additional columns may be purchased from Amersham Biosciences (#17-0851-01).

D. Use of Spin Columns

We recommend that the largest volume of antibody solution reduced at one time not exceed 60 µl. Lower volumes reduce antibody recovery; higher volumes risk DTT breakthrough. If using Spin Column to exchange the conjugate into Storage Buffer, antibody solution should not exceed 100 µl.

j. Spin Column must be equilibrated with the appropriate buffer before use (Exchange Buffer for Antibody Reduction or Storage Buffer for Conjugate Finishing):
k. Snap off the tip, remove the cap, and place the tip of the column into the capless 2-ml Wash Tube.
l. Swirl column gently or flick with a fingertip to set the bed evenly in the column.
m. Allow the excess packing buffer to drain by gravity to the top of the gel bed; discard buffer.
n. If column does not begin to flow, push the cap back onto column and remove again, this should break the surface tension. Alternatively, centrifuge for a short time.
o. Fill column (~1.5 ml) with appropriate buffer.
p. Centrifuge the assembly for 4 minutes in a clinical or other centrifuge with a swinging bucket rotor capable of generating a force of 1000 x g.
q. Discard buffer from the Wash Tube.
r. Add buffer, centrifuge and discard again.
s. Without adding additional buffer, spin the column for 4 minutes to ensure complete removal of extraneous buffer. Little or no additional buffer (<50 µl) should drain into the collection tube.

NOTE: Handle the column gently as the bed can be easily disrupted.

NOTE: The column should appear dry and compact, and sit evenly in the column. If channels or excess air are evident, add a small amount of buffer and spin briefly to reset the column bed.

The Spin Column should not be reused. Additional columns may be purchased from Bio-Rad Laboratories (#732-6002).

E. Use of Preservatives

Buffers and other reagents included in this kit contain pentachlorophenol (PCP) or sodium azide as preservatives. Conjugates prepared and stored in buffers containing PCP do not adversely affect living cells when diluted appropriately for use in flow cytometry applications. Sodium azide at 0.02 - 0.05% is preferable if your application allows it; it may be added directly to PCP-containing buffers.

If preservatives must be omitted, buffer may be prepared without adding PCP (Appendix B, page 24). Similarly, Desalting Column B has been equilibrated with buffer containing 0.5 µg/ml of PCP. The column must be re-equilibrated with buffer that does not contain PCP if you do not wish to include it in your final conjugate.

NOTE: Conjugates which do not contain preservative should be filter-sterilized and stored aseptically to prevent microbial contamination.

F. Troubleshooting Poor or Failed Conjugations; TECHNICAL ASSISTANCE

Activated phycobiliproteins have been tested extensively in customer applications. If you have any questions or experience difficulties regarding any aspect of our products, please contact us:

Phone help line: 443-430-0403
(Toll Free) 866-568-5772
Fax: 443-430-0407
e-mail: techsupport@columbiabiosciences.com

Our customers are an important source of information regarding advanced or specialized uses of our products. We encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

APPENDIX A: SPECTRAL PROPERTIES & SPECIFICATIONS

PRODUCT: SureLight® Allophycocyanin
BUFFER: 100 mM SPEA pH 7.0
PRESERVATIVE: 0.05% sodium azide
STORAGE: Once reconstituted, store at 4°C in the dark. DO NOT FREEZE.

Purity:
Absorbance @ 650 nm/Absorbance @ 280 nm ≥ 4.6
Absorbance @ 650/Absorbance @ 620 nm ≤ 1.5
Please contact us for a lot-specific Certificate of Analysis or Assay Protocols

Absorbance Spectrum: (peak location at 650 nm)

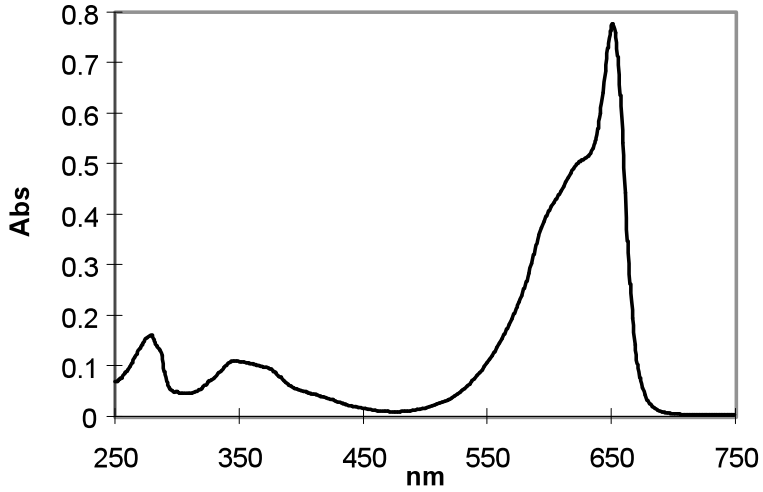

Figure 1. Absorbance spectrum of 0.1 mg/mL in 100 mM sodium phosphate (pH 7.0) and 0.05% sodium azide.

Fluorescence (normalized to absorbance, excitation 650 nm, emission 662 nm)

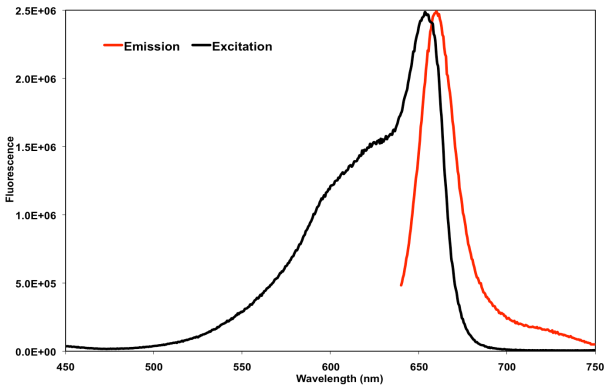

Figure 2. Normalized fluorescence emission and excitation spectra of sample at 0.167 µg/mL in 100 mM sodium phosphate (pH 7.2) and 0.05% sodium azide.

APPENDIX B: COMPOSITION OF BUFFERS

Dialysis Buffer

50 mM sodium phosphate, 1 mM EDTA (pH 7.0)

To make 1 liter:
6.0 g sodium phosphate monobasic (anhydrous)
0.37 g EDTA (disodium)
Adjust to pH 7.0 with NaOH

Exchange Buffer

50 mM MES, 2 mM EDTA (pH 6.0)

To make 1 liter:
9.8 g MES (2-[N-morpholino] ethanesulfonic acid)
0.74 g EDTA (disodium)
Adjust to pH 6.0 with NaOH

Storage Buffer

10 mM Tris, 150 mM NaCl (pH 8.2) and 0.5 g/ml pentachlorophenol

To make 1 liter:
1.2 g Tris base
8.8 g NaCl
Adjust to pH 8.2 with HCl
If desired, add 0.5 ml of a 1 mg/ml stock solution of pentachlorophenol (in ethanol) to make 0.5 µg/ml

APPENDIX C: DETERMINATION OF PROTEIN CONCENTRATION

The concentration of protein in the Desalting Column fractions can be determined spectrophotometrically by measurement of the absorbance at 280 nm:

1. Read the Absorbance of each fraction directly (without dilution) at 280 nm.
2. Pool the fractions that comprise the bulk of the 280 nm peak.
3. Proceed immediately to the Covalent Conjugation step.

Alternatively, a simple colorimetric assay may be performed:

1. Place 2 µl aliquots of Bradford Reagent on wax paper or parafilm.
2. Add 8 µl aliquots from each fraction to the reagent droplets and mix. Protein-containing droplets will turn blue.
3. Pool the fractions containing the majority of the reduced IgG.
4. Proceed immediately to the Covalent Conjugation step.

Bradford reagent is available from Bio-Rad Laboratories (#500-0006) or Sigma-Aldrich (#B-6916).

APPENDIX D: SOURCE FOR MATERIALS

Activated SL-APC may be purchased separately (from Columbia) for greater flexibility when using this kit.

Other kit components and suggested reagents may be purchased directly from their manufacturers:
Desalting Columns (A and B) - PD-10 Columns, Sephadex™ G-25 M, Amersham Biosciences (#17-0851-01)
Ultrafilter – Ultrafree® 0.5 Centrifugal Filter Device with Biomax™-30 membrane, Millipore Corporation (#UFV5 BTK)
Spin Column - Bio-Spin® 6 Chromatography Columns with 2 collection tubes, Bio-Rad Laboratories (#732-6002)

TRADEMARKS

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Bio-Spin® is a registered tradename of Bio-Rad Laboratories
Sephadex™ is a trademark of Amersham Biosciences
SureLight® is a trademark of Columbia Biosciences