PROzyme®

GLYKO®

PHYCOLINK® R-PHYCOERYTHRIN (RPE) CONJUGATION KIT

All reagents needed to conjugate 1 mg of antibody or other protein

Complete protocols, thoroughly tested for trouble-free conjugations in ~2.5 hours

Configured to enable conjugations with as little as 50 µg of antibody

Pre-activated RPE for simple, easy conjugations

Brightest RPE commercially available for more sensitive conjugates

Activated RPE also sold separately (PJRC10) to allow flexibility when using kit components

Product Code PJ31K

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KIT CONTENTS

Item	Qty
PhycoLink Activated RPE [enough for 1 mg of antibody]	3.2 mg
Dithiothreitol (DTT) Stock Solution [1 *M*]	0.1 ml
Blue Dextran Stock Solution [50 mg/ml, 25x]	0.1 ml
N-ethylmaleimide (NEM) Stock Solution [10 mg/ml]	0.1 ml
Exchange Buffer	40 ml
Storage Buffer	40 ml
Desalting Column A	1
Desalting Column B	1
Desalting Column Collection Tube	2
Ultrafilter (C) plus Filtrate Collection Tube	2
Spin Column (D) plus Wash Tube and Collection Tube	2

ITEMS NOT SUPPLIED

Required

Microcentrifuge and Tubes

Optional

Stirring Motor and Dialysis Buffer for Dialysis
Clinical Centrifuge for Spin Columns
Rotator or Slow Mixer for Conjugation
Centrifuge for Ultrafilters
Spectrophotometer for Absorbance Measurements

STORAGE CONDITIONS

PhycoLink 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.

RPE solutions and Desalting/Spin Columns should not be frozen.

For optimal performance, DTT and NEM Stock Solutions should be stored at -20°C if this kit will not be used within 30 days.

The final conjugate should be protected from light and stored at 4°C; DO NOT FREEZE.

REFERENCES

Technical literature referred to in the text may be found on ProZyme's website:

FAQ's:

http://www.prozyme.com/faqs.html

TechNotes:

http://www.prozyme.com/tech_notes.html

INTRODUCTION

PhycoLink Activated R-Phycoerythrin (RPE) is a highly fluorescent phycobiliprotein which has been chemically activated for easy conjugation to reduced immunoglobulins or other sulfhydryl-containing proteins. The RPE 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 RPE 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 RPE are stable at 4°C for extended periods of time, both before and after conjugation. Although the number of maleimide groups on Activated RPE will decline slowly over time, its performance in standard antibody conjugation assays is not substantially reduced after as much as a year at 4°C in Exchange Buffer.

The conjugation protocol is optimized for site-specific conjugation of RPE to antibodies with these advantages:

- a well-defined procedure
- no interference with the antigen binding site
- robust results, *i.e.* good conjugates over a range of antibody concentrations

The protocol is written to conjugate 1 mg of antibody using Desalting Columns A and B. Customers report good results with quantities as low as 0.25 mg of antibody. Sometimes, however, antibody supplies are limited or cost-prohibitive. Ultrafilters (C) and Spin Columns (D) are included to enable smaller conjugations (as low as 50 µg) that approximate the same reaction conditions. Suggestions for use of Desalting Columns, Ultrafilters and Spin Columns are included in the TIPS & HINTS section in this booklet (pp. 21 - 25).

The conjugation protocol is divided into four steps (see Protocol section for details):

1. Antibody Preparation - Purified antibody at 1 - 10 mg/ml should be formulated in a buffer which will not interfere with DTT reduction.

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

3. Covalent Conjugation - Activated RPE 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 RPE 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.

4. 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.

The protocols do not include a purification step for removal of the unreacted RPE. 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 often required (see pp. 9 - 10).

Kit Flexibility

The Desalting Columns (A and B) and Spin Columns (D) 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 (D) 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 a different conjugation.

3. Perform two small-scale conjugations of different antibodies using the two Spin Columns (D) to desalt the reduced antibodies. The two Ultrafilters (C) 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 an additional larger-scale conjugation.

4. Perform three medium-scale conjugations (0.25 - 0.5 mg) with different antibodies: the two Spin Columns (D) and Desalting Column A can each be used to desalt one reduced antibody. Then Desalting Column B can be used for buffer exchange of one conjugate, and subsequently one or both of the desalting columns can be re-equilibrated with Storage Buffer and be used for buffer exchange of the other two conjugates. Alternatively, 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 RPE (PhycoLink PJRC10 Activated RPE) 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 RPE. With some antibodies, there may be little difference between the conjugates produced at low and high ratios of RPE:antibody, but with others a molar ratio as low as 1:1 can result in some or all of the conjugate precipitating. The high ratio of RPE to antibody in the protocol minimizes this tendency.

When scaling up conjugations, it is important to maintain similar reaction conditions. We have found that 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

PhycoLink Conjugation Kits are designed for easy conjugation of antibodies and other proteins to phycoproteins (fluorescent proteins including phycobiliproteins and PerCP) and their tandems. Conjugates produced with these kits contain unincorporated phycoprotein (since it is supplied in excess), and may also contain small amounts of unincorporated antibody. The PhycoLink Purification Kits (product codes KPK13 and KPK80, available from ProZyme) provide the means to remove unincorporated reactants.

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. Losses of over 50% are possible when processing small quantities and should be considered before adding this step.

Special Applications

Customers have reported successful conjugations with various IgMs using this protocol. Other sulfhydryl-containing molecules besides immunoglobulins may also be conjugated using this protocol.

Molecules which do not contain available sulfhydryls may be conjugated if they are first treated with a reagent to introduce sulfhydryls via primary amines. Suggested protocols for alternate conjugation methods are described in TechNote TNPJ300 *Alternative Conjugation Protocols*.

PROTOCOL

NOTE: We want successful conjugations for our customers, so please read this entire section as well as the TIPS & HINTS section (pp. 21 - 25) before beginning.

Antibody Preparation (0 - 16 Hours)

Time requirement varies depending on procedure used.

Reagents

Purified antibody solutions at 1 - 10 mg/ml (4 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 concentrating the antibody if its concentration is less than 1 mg/ml. Dilute antibody solutions may be concentrated using the Ultrafilter (C), provided with the kit (see Use of Ultrafilters to Concentrate Antibody Solutions in the TIPS & HINTS section, page 21).

Reaction Size

As little as 50 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.

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.

Antibody Reduction (~40 Minutes)

Two alternate procedures are given to prepare the reduced antibody for conjugation. The Spin Column Procedure is preferable for small-scale conjugations because it does not result in dilution of the antibody, but is limited to sample volumes of 50 - 60 µl of reduced antibody. In contrast, the Desalting Column Procedure accommodates antibody sample volumes of up to 1.7 ml. In general, choose the Desalting Column Procedure for conjugations of ≥0.5 mg of antibody, or choose the Spin Column Procedure for conjugations of ≤0.25 mg. For 0.25 - 0.5 mg conjugations, the choice of procedure will depend on the antibody volume.

Reagents

Antibody Solution

DTT Stock Solution (supplied with kit)

Blue Dextran Stock Solution (supplied with kit, optional in Desalting Column Procedure)

NOTE: Be careful to return caps to the proper vials. Match colored dots on caps and vials.

Exchange Buffer (supplied with kit)

Either Desalting Column A or Spin Column D (supplied with kit; see separate procedures below)

Desalting Column Procedure

NOTE: See Use of Desalting Columns in the TIPS & HINTS section (pp. 22 - 23).

Add 20 µl of DTT Stock Solution per ml of antibody solution and mix. Example: for 1 mg of antibody at 4 mg/ml, add 5 µl of DTT Stock Solution to 250 µl of antibody solution.

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

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

Optional: Add 40 µl of Blue Dextran Stock Solution per ml of reduced antibody solution and mix. In the example given above (250 µl of antibody at 4 mg/ml), add 10 µl of Blue Dextran Stock Solution.

NOTE: See Use of Blue Dextran in the TIPS & HINTS section (page 22).

Drain the excess buffer from Column A (supplied pre-equilibrated in Exchange Buffer) before applying the reduced antibody sample.

Apply the reduced antibody to Desalting Column A, then follow with Exchange Buffer. The antibody will begin to elute after a total of ~2.5 ml (sample plus buffer) have entered the column. After this, the antibody will elute within the next ~3 ml of eluate. Do not collect past this volume, or else the antibody may be contaminated with DTT.

NOTE: For best separation, the volume of reduced antibody sample loaded onto the Desalting Column should be no more than 1.7 ml.

If Blue Dextran is present, begin collecting material as soon as blue color appears in the drop. Stop collecting when about 90% of the blue color has eluted from the column. This usually means a collected volume about 0.5 ml larger than the volume loaded.

If Blue Dextran has not been added, collect the reduced antibody by one of the following two methods:

1) Collect 0.25 - 0.5 ml fractions and determine the protein content (see Appendix C). Pool the fractions containing the majority of the reduced antibody.

2) Perform a batch elution. After applying the reduced antibody to the column, follow with sufficient Exchange Buffer such that the total volume of sample plus buffer equals 2.5 ml. Discard the eluate up to this point. Then add 2.5 ml of Exchange Buffer to the column and collect the eluate as a single fraction, which will contain the reduced antibody.

NOTE: Method 2 will result in a lower protein concentration than either method 1 or through the use of Blue Dextran. Accordingly, method 2 should only be used for a relatively large-scale conjugation (i.e. *at least 0.5 mg of antibody).*

Proceed to Covalent Conjugation as soon as possible because the antibody sulfhydryls will re-oxidize.

Spin Column Procedure

NOTE: See Use of Spin Columns in the TIPS & HINTS section (pp. 23 - 24).

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

Add 1 µl of DTT Stock Solution.

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

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

Equilibrate Spin Column D with Exchange Buffer and drain (as described on pp. 23 - 24).

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

Centrifuge for 4 minutes at 1000 x g. The eluate 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.

Proceed to Covalent Conjugation as soon as possible because the antibody sulfhydryls will re-oxidize.

Covalent Conjugation (~80 Minutes)

Reagents

PhycoLink Activated RPE (supplied in kit)

NEM Stock Solution (supplied in kit)

Procedure

Add 3.2 mg (320 µl) of Activated RPE per mg of antibody (assume 100% recovery).

NOTE: If the reaction volume is <1.5 ml, use a small tube such as a microcentrifuge tube to minimize oxygenation.

Wrap the reaction tube in aluminum foil to protect the reaction from light and incubate for 60 minutes at room temperature. *Optional: use a rotator or other slow mixer.*

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

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

NOTE: It is acceptable to be approximate with the actual amount of NEM since it is added in substantial excess.

Wrap and incubate again for 20 minutes at room temperature.

Proceed to the next step promptly, if possible. If not, the conjugate may be stored in the refrigerator as-is for up to a few days.

If the conjugate is to be purified to remove unreacted proteins, skip Conjugate Finishing and go directly to the purification procedure (purification kits are available from ProZyme under product codes KPK13 and KPK80).

Conjugate Finishing (10 Minutes - 16 Hours)

Time requirement varies depending on procedure used.

Two alternate procedures are given to exchange the conjugate into a buffer suitable for storage and to remove residual NEM and DMSO. The Spin Column Procedure is preferable for small volumes because it does not result in dilution of the conjugate; it is limited to sample volumes of ≤100 µl. The Desalting Column Procedure is used for all samples >100 µl.

NOTE: Keep the final conjugate at high concentration (>1 mg/ml) for optimal stability. For long-term storage of very dilute conjugates, it may be advisable to concentrate the final conjugate by ultrafiltration after the final desalting step. Be aware, however, that ultrafiltration will result in loss of some of the material (particularly for small-scale conjugations and low-protein concentrations), so this step should be avoided if possible.

Reagents

Storage Buffer (supplied with kit)

Either Desalting Column B or Spin Column D (supplied with kit, see separate procedures below)

Ultrafilter C (supplied with kit, optional)

Desalting Column Procedure

NOTE: See Use of Desalting Columns in the TIPS & HINTS section (pp. 22 - 23).

Drain the excess buffer from Column B before applying the conjugate.

Spin the conjugate in a microcentrifuge for about 30 seconds to pellet insoluble conjugate aggregates that might clog the column frit. Remove the supernatant and apply it to the column. Discard the pellet.

Using the Storage Buffer supplied in the kit, pass the conjugate over Desalting Column B (supplied pre-equilibrated in Storage Buffer).

Begin collecting the conjugate when the first color appears in the effluent. Stop collecting when almost all of the color (~95%) has eluted from the column.

NOTE: The conjugate can be exchanged instead into another appropriate buffer using Column B, provided that the column is first equilibrated by passing 20 - 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.

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

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

Spin Column Procedure

NOTE: See Use of Spin Columns in the TIPS & HINTS section (pp. 23 - 24).

Start with up to 100 µl of conjugate solution. If volume is <50µl, add buffer to a final volume of 50 - 100 µl.

Spin the conjugate in a microcentrifuge for about 30 seconds to pellet insoluble conjugate aggregates that might clog the column. Remove the supernatant and apply it to the column. Discard the pellet.

Equilibrate Spin Column D with Storage Buffer and drain (as described on pp. 23 - 24).

Place the tip of Spin Column D into the Collection Tube (standard 1.5 ml Eppendorf tube supplied with Spin Column D). Carefully apply up to 100 µl of the conjugate directly to the center of the column.

Centrifuge for 4 minutes at 1000 x g. The eluate 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.

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

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

TIPS & HINTS

Consult the FAQs on ProZyme's website for the answers to common questions.

Use of Ultrafilters to Concentrate Antibody Solutions

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

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

Pour or pipette up to 0.5 ml antibody solution into the Ultrafilter (C). Close the cap tightly.

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. Spin at up to 10,000 x g, checking periodically until the desired final retained volume (50 - 60 µl) is obtained.

Transfer the concentrated antibody solution into a microcentrifuge tube.

The Ultrafilter should not be reused. Additional units may be purchased directly from Millipore Corporation (#UFC503096).

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.

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.

Care should be taken when loading samples to minimize dilution. 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.

Columns may be reused by washing with at least 25 ml of appropriate buffer. Additional columns may be purchased directly from GE Healthcare (#17-0851-01).

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 D to exchange the conjugate into Storage Buffer, the antibody solution should not exceed 100 µl.

Spin Column D must be equilibrated with the appropriate buffer before use (Exchange Buffer for Antibody Reduction or Storage Buffer for Conjugate Finishing):

Snap off the tip, remove the cap, and place the tip of the column into the capless 2-ml Wash Tube. Swirl column gently or flick with a fingertip to set the bed evenly in the column. Allow the packing buffer to drain by gravity to the top of the gel bed; discard buffer.

NOTE: 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.

Fill column (~1.5 ml) with appropriate buffer.

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.

Discard buffer from the Wash Tube.

Add buffer, centrifuge and discard again.

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 directly from Bio-Rad Laboratories (#732-6002).

Use of Preservatives

Buffers and other reagents included in this kit contain pentachlorophenol (PCP) as a preservative. 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, pp. 29 - 30). Similarly, Desalting Columns A and B have been equilibrated with buffers containing 0.5 µg/ml of PCP. The columns 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.

Troubleshooting Poor or Failed Conjugations

Consult the FAQs on ProZyme's website for the answers to common questions. If you cannot find an applicable entry, please fill out the questionnaire provided as an FAQ:

http://www.prozyme.com/faqs/pjg0001.html

Fax or e-mail your answers to us, and we will respond with comments and suggestions.

TECHNICAL ASSISTANCE

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

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WEB **www.prozyme.com**

ProZyme 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.

Or, contact your local distributor. A list of ProZyme's distributors, with contact information, may be found at:

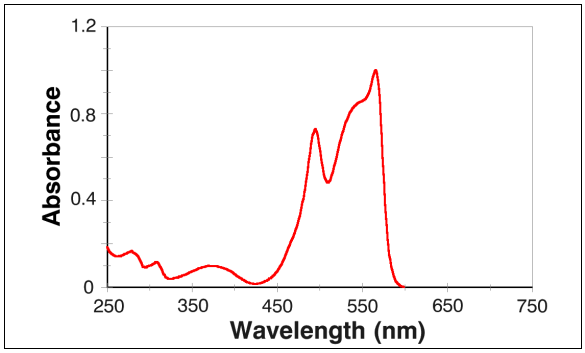
http://www.prozyme.com/distributors.html

APPENDIX A: SPECTRAL PROPERTIES & SPECIFICATIONS

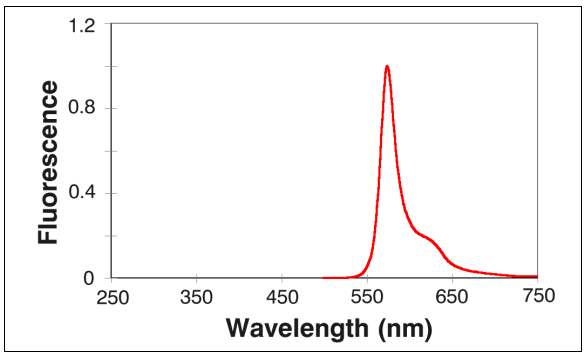
PRODUCT:	Activated RPE
BUFFER:	50 m*M* MES, 2 m*M* EDTA (pH 6.0)
PRESERVATIVE:	1 µg/ml pentachlorophenol
STORAGE:	Store at 4°C in the dark. DO NOT FREEZE.
PURITY:	$A_{566}:A_{280} > 5.0$

Please contact us for a lot-specific Certificate of Analysis or Assay Protocols

Absorbance Spectrum: (peak location at 566 and 496 nm)



Fluorescence Emission Spectrum: (arbitrary units, peak location at 575 nm)



APPENDIX B: COMPOSITION OF BUFFERS

Dialysis Buffer

50 m*M* sodium phosphate, 1 m*M* 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 m*M* MES, 2 m*M* EDTA (pH 6.0) and 0.5 µg/ml pentachlorophenol

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
If desired, add 0.5 ml of a 1 mg/ml stock solution of pentachlorophenol (in ethanol) to make 0.5 µg/ml

Storage Buffer

10 m*M* Tris-HCl, 150 m*M* NaCl (pH 8.2) and 0.5 µg/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 (requires a microcuvette):

Read the A_{280} of each fraction directly (without dilution).

Pool the fractions that comprise the bulk of the peak.

Proceed immediately to the Covalent Conjugation step.

Alternatively, a simple colorimetric assay may be performed:

Place 2 µl aliquots of Bradford Reagent on wax paper or Parafilm.

Add 8 µl aliquots from each fraction to the reagent droplets and mix. Protein-containing droplets will turn blue.

Pool the fractions containing the majority of the reduced antibody.

Proceed immediately to the Covalent Conjugation step.

APPENDIX D: SOURCE FOR MATERIALS

PhycoLink PJRC10 Activated RPE may be purchased separately 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, GE Healthcare (#17-0851-01)

Ultrafilter (C) - Amicon® Ultra-0.5 Centrifugal Filter Unit with Ultracel®-30 membrane , Millipore Corporation (#UFC503096)

Spin Column (D) - Bio-Spin® 6 Chromatography Columns with 2 collection tubes, Bio-Rad Laboratories (#732-6002)

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

OTHER PROZYME PRODUCTS & KITS

ProZyme offers a variety of fluorescent labeling reagents and kits. A complete listing may be found on our website at:

http://www.prozyme.com/

PRODUCT USE AND WARRANTY

Terms and conditions of sale may be found at:

http://www.prozyme.com/pdf/terms.html

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Sephadex™ is a trademark of Amersham Biosciences

PLACING AN ORDER

For North American destinations: telephone orders may be placed between 8:00 am and 5:00 pm Pacific Time. Telefax or e-mail orders may be sent or messages recorded anytime.

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