User Guide

Version 1.0 (April 2007)

Product: **Platinum*Link*™ Antibody Labeling Kit**
Catalog number (s): PLK-007, PLK-008, PLK-009, PLK-010
Lot number: See label on package
Unit Size: 4 labeling reactions (100µg antibody per labeling)

For the full manual please visit www.kreatech.com

A. Components & Storage - IMPORTANT

All components should be stored at 2-6ºC upon arrival.

Component	Description of function	Amount
10 x PL buffer	10 x Protein Labeling buffer	40µl
Label: BIO-ULS, or DNP-ULS, or FLU-ULS, or RHO-ULS	Labeling reagent	20µl
ULS-Trap columns	Column for removal of non-reacted ULS	4
Collection tubes	Use in combination with ULS-Trap columns	8

Materials needed but not Included in Kit
1.5 ml reaction vials (*e.g.* Eppendorf), Table top microcentrifuge (*e.g.* Eppendorf), 37 ºC incubator or waterbath, Purified water (*e.g.* MilliQ), Antibody of interest

B. General Information

KREATECH's Platinum*Link* Kits provide a convenient way to conjugate biotin (BIO), dinitrophenol (DNP), fluorescein (FLU) or rhodamine (RHO) to antibodies (or other proteins). The kits contain all components necessary to perform four separate labeling reactions including subsequent removal of non-reacted label using ULS-Trap columns. The standard protocol is optimized for the labeling of 100 µg antibody at a final concentration of 1 mg/ml during labeling.
Other proteins like recombinant proteins or Fab'-fragments can be labeled as well. In general, following this standard protocol a labeling degree of 2-4 is obtained. **For more information please refer to the full manual that can be downloaded from our website: www.kreatech.com.**

1. ULS Labeling of Antibodies

The labeling protocol described here has been optimized for the labeling of 100 µg of antibodies in a 100 µl labeling volume. For example, 10 µl of a 10 mg/ml stock solution is added to the 100 µl labeling volume (or 20 µl of a 5 mg/ml stock, 50 µl of a 2 mg/ml stock, etc). If the protein concentration of your antibody stock solution is lower than 1.18 mg/ml, addition of 100 µg antibody to a 100 µl end volume will not be feasible. In that case, consider concentrating your antibody stock before labeling.

Alternatively, add as much antibody as possible. When the protein concentration in the labeling mixture is ≤ 0.5 mg/ml, the amount of label has to be adjusted according to Table 1 in order to prevent over-labeling

Protein concentration in labeling mixture	Amount of ULS-label needed
1 mg/ml	5 µl
0.5 mg/ml	3 µl
0.1 mg/ml	1 µl

Table 1: Amount of ULS-label needed when using lower protein concentrations in the labeling mixture.

With the protocol described in this manual we have obtained very good results without using protease inhibitors. However, if protease inhibitors are required during labeling, Roche Complete™ has been found to be compatible with ULS-labeling.

C. Protocol

Please read the entire protocol before starting

- Before use, shortly centrifuge all vials to collect fluids at the bottom of the vial.
- BIO-ULS, DNP-ULS and RHO-ULS come as aqueous solutions. FLU-ULS comes as a suspension; prior to use thoroughly resuspend the FLU-ULS by pipetting.

OPTIONAL: Sample preparation

- Generally, purification of antibody stocks prior to ULS labeling is not necessary. However, in case your antibody stock solution contains incompatible substances as described on page 9 of the full manual, buffer exchange is recommended.
- In case the protein concentration of your antibody stock is not known, determine protein concentration using for instance a BCA Protein Assay Kit (*e.g.* Pierce # 23225). Follow the manufacturer's instructions.

I. ULS labeling of antibodies

- Prepare one 1.5 ml Eppendorf vial for each labeling reaction (vials not provided).
- Add the reagents in the order as is shown in Table 2.

NOTE: if antibody stock concentration is lower than 0.5 mg/ml, the amount of label has to be adjusted according to Table 1.

Component	Volumes
Antibody of interest	100 µg (*e.g.* 10 µl of 10 mg/ml stock)
Purified water	Adjust volume to 85 µl
10x PL buffer	10 µl
BIO-ULS **or** DNP-ULS **or** FLU-ULS **or** RHO-ULS	5 µl
Total volume	**100 µl**

Table 2: antibody labeling with ULS labels

- Thoroughly mix the samples by pipetting and incubate overnight (16 hrs) at 37°C in a water bath or incubator.

Optional: the samples may be incubated at 50 ºC for four hours to reduce labeling time.

II. Free label removal using ULS-Trap columns

Preparing the ULS-Trap column

Prepare one ULS-Trap column for each 100 µl labeling reaction:

- Invert the column several times to resuspend the content
- Snap off the bottom of the column and **remove the cap**
- Place the column in a 2 ml polypropylene sample collection tube
- Spin column dry: 1 minute at maximum speed (~20,000 g) in a table top microcentrifuge
- Discard the 2 ml collection tube together with its content
- Place the column in a new 2 ml polypropylene sample collection tube

Removal of free label

- Load 100 µl labeled sample onto the center of a ULS-Trap Column.
- Centrifuge column with sample for 1 minute at maximum speed (~20,000 g) in a table top microcentrifuge; the flow through contains the labeled antibody, non-reacted ULS is retained on the column.
- Transfer your purified, labeled antibody to a storage vial (not provided).
- If desired, desalt the solution and/or add preservative (*e.g.* EDTA, glycerol, carrier protein)

III. Determination of the degree of labeling

There may be occasions that one wishes to check the degree of labeling of the antibodies. This is done by measuring the absorbance of DNP, FLU or RHO and calculating the amount of ULS molecules using the molar extinction coefficient. For BIO this is not possible, but instead, biotin might be determined using the HABA/avidin method (Pierce, Cat no # 28010).

Essential to both methods is that after the labeling reaction the excess of free label is removed.

Determination of the degree of labeling for DNP, FLU or RHO labeled antibodies:

- Label the antibody as described
- Remove non-reacted ULS label using ULS-Trap column
- Perform a protein concentration assay on a fraction of the labeled and purified material. In contrast to the protein concentration determination prior to labeling, we now recommend using Bio-Rad's Rc Dc protein assay (Bio-Rad # 500-0120), since the BCA assay is not compatible with antibodies purified over ULS-Trap columns.
- Measure absorbance in a spectrophotometer at the wavelength indicated in Table 3.
- Calculate the degree of labeling using the following formula:

$$\text{label/ antibody} = \frac{A \times MW}{\varepsilon \times c \times l}$$

where:

A: absorbance at indicated wavelength
ε: molar extinction coefficient as given in Table 3
l: cuvette path length (cm)
c: protein concentration in mg/ml
MW: molecular weight of the antibody in g/mol

Label	Absorbance maximum (nm)	Emission maximum (nm)	Extinction coefficient ($l\ mol^{-1}\ cm^{-1}$)	Solvent
DNP-ULS	363	N.A.	18,000	MeOH
FLU-ULS	495	517	83,000	pH 9
RHO-ULS	550	573	91,000	MeOH

Table 3 : Spectral characteristics of labels

Determination of the degree of labeling for biotinylated antibodies:

- Label the antibody as described
- Remove excess BIO-ULS™ using ULS-Trap column
- Perform a protein concentration assay on a fraction of the labeled and purified material (eg using Bio-Rad # 500-0120).
- Determine the molar ratio of biotin incorporated into a antibody using the HABA-Avidin method. We recommend to use HABA from Pierce (catalogue #28010) following the manufacturer's instructions.

For **Trouble Shooting** and **References** please refer to the full manual on our website www.kreatech.com

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