

# Linkit<sup>TM</sup>

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Linkit<sup>TM</sup> Bio-Link (Code BL-100)



**BIO-LINK**  
**ANTIBODY**  
**&**  
**PROTEIN**  
**BIOTINYLATION**  
Instruction Protocol  
1-10mg  
PRODUCT CODE BL100

## PROTOCOL CONTENTS

- A. G50 Buffer Exchange Columns, x 3. Store at +4°C - +25°C. **HAZARDOUS**, contains 0.05% sodium azide as a preservative during shipment. Avoid contact with skin and eyes or swallowing. In the event of contamination, seek immediate medical advice.
- B. Biotinylation Buffer, 1 x 125ml. Store at +4°C - +25°C.
- C. Reaction Tube, x 3. Store at +4°C - +25°C.
- D. Storage Buffer, 1 x 250ml. Store at +4°C - +25°C. **HAZARDOUS**, contains 0.05% sodium azide as a preservative during shipment. Avoid contact with skin and eyes or swallowing. In the event of contamination, seek immediate medical advice.
- E. Solvent for Biotinylation Reagent, 3 x 1.25ml vials. Store at +4°C - +25°C.
- F. Biotin Labelling Reagent, 3 vials. Store at -20°C.
- G. Collection Tube, 3 tubes. Store at +4°C - +25°C.

**Store Biotin Labelling Reagent at -20°C.**

**Store the remaining kit at +4 to +25°C, but do not freeze.**

If any packs are damaged or bottles appear to have leaked, do not use the items, but contact ISL for advice.

This kit is supplied for Research use only. ISL will not accept responsibility for misuse of the Protocol components. This kit contains glass items which should be handled with due care.

## INTRODUCTION

1. The Protocol contains sufficient reagents for 3 antibody labellings, though it can equally be used for other complex proteins of >75kD (Additional formats are available for low molecular weight proteins or antibody fragments).
2. Any quantity of antibody between 1 and 10mg can be labelled with this kit. This kit is also suitable for antibodies prepared with stabilising proteins such as Bovine Serum Albumin. The BSA will also be labelled but should not affect the reactivity of the antibody for the antigen.
3. The labelling procedure takes 5 hours to complete. First the antibody preparation is transferred to biotinylation buffer. The biotin label is added and the reaction allowed to proceed for 4 hours at room temperature. The biotinylated antibody preparation is then transferred to phosphate buffered saline containing 0.1% sodium azide preservative, for storage and subsequent use.

## THE PROTOCOL

**You are advised to wear safety glasses and gloves during and after use of the Biotinylation Reagents and Solvent.**

- A. Exchange to Biotinylation Buffer**
  1. Unpack one filtration column (**A**). Remove the upper cap and pour off the excess buffer. Place the column vertically in a clamp stand, remove the outlet cap and allow any excess buffer to drain to waste.
  2. Add 20ml of biotinylation buffer (**B**) to the column and allow the column to drain to waste. The flow rate should be approximately a drip/sec. If it is considerably slower than this the column outlet may be partially obscured and should be carefully trimmed with scissors or a sharp blade.
  3. Add 3ml of antibody sample to the column and allow the column to drain to waste.
  4. Place the reaction tube (**C**) at the column outlet to collect the antibody as it is eluted. Add 4.5ml biotinylation buffer (**B**) to the column and collect the sample in the reaction tube.
  6. Remove the reaction tube. Add 20ml storage buffer to the reservoir of the column (**D**) and allow to drain whilst continuing with the conjugation. Cap the outlet with the yellow closure supplied once the column has drained.

### **B. Biotin Labelling**

The following protocol gives an approximate molar ratio of 20 biotins to each antibody. However, as the reaction site for the biotin occurs in the antibody at Lysine amino acid residues, the antibody to target reactivity of some antibodies may be reduced by this degree of labelling. The biotin to antibody ratio may be changed, see the notes at the end of the protocol for guidance.

1. Immediately before use, open one vial of solvent (**E**), wear gloves for safety. This reagent may solidify at temperatures of < 15°C and should be warmed gently until melted.
2. Using a micropipette, transfer 1ml of the solvent to the vial containing the biotinylation reagent (**F**).
3. Place the vial containing the antibody on a magnetic stirrer. For each milligram of antibody in the sample to be labelled, add drop-wise 100µl of the dissolved biotinylation reagent to the antibody sample in the reaction vial (**C**) whilst stirring gently.
4. Re-cap the reaction tube and continue to stir the reaction mixture gently for 4 hours at room temperature.

### **C. Exchange to Storage Buffer**

1. Make up the reaction volume to 6ml by addition of storage buffer. Add half (i.e. 3ml) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.
2. Place the collection tube (**G**) at the column outlet to collect the biotinylated antibody. Add 4.5ml storage buffer to the column and collect the eluted sample in the collection tube.
3. Add 20ml storage buffer to the column and drain to waste.
4. Add the remainder of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.
5. Place the collection tube (**H**) at the column outlet to collect the biotin labelled antibody. Add 4.5ml storage buffer to the column and collect the eluted sample.
6. Store the biotinylated antibody preparation at 4°C.

## Other Antibody Labelling Kits

### Protocol Notes

Note 1: Quantity of antibody.

The kit is intended for labelling of 5mg of antibody. However, any quantity of antibody in the range 1-10mg can be used successfully, simply by adjusting the antibody concentration and altering the proportion of biotinylation reagent added. However, it is important not to exceed the *volumes* indicated as this will give losse and inefficient buffer exchange.

Dialysis may be used at section C. This is however a lengthy alternative and will require 3 x 8 hours of dialysis against fresh 1 litre amounts of buffer.

Note 2: To change the Biotin linking ratio.

The biotin to antibody linking ratio may be changed, if required, by altering the volume of biotin label added at stage B3 above.

To reduce the ratio, add a smaller volume of biotin label and simply follow the protocol instructions.

Greater biotin to antibody labelling ratios can also be produced by addition of up to 1.25ml of biotin label at stage B3.

However, as the reaction site for the biotin occurs in the antibody at Lysine amino acid residues, there is a limit to the level of biotinylation that can be achieved and particularly for monoclonal antibodies, this will vary between different antibodies. Increasing the conjugation ratio may reduce antibody reactivity.

#### Peroxi-Link

Horse-radish-Peroxidase

Product code ISPT. 3 separate 5mg antibody labellings.

£ 149.00

Horse-radish peroxidase is the first choice visualisation reagent for many laboratories. Development reagents are available to give a variety of colours in aqueous form (for use with ELISA), or permanent pigment deposited *in situ* (immunohistology or protein immunoblotting techniques). Substrates are also available for using this enzyme system with highly sensitive chemiluminescence systems.

#### Phospha-Link

Alkaline Phosphatase

Product code ISPR. 3 separate 5mg antibody labellings.

£ 149.00

A wide variety of detection systems is available for this enzyme system. The kit uses calf intestinal alkaline-phosphatase, which has significant advantages over the other isoenzymes in that it can be selectively inhibited.

#### Gluco-Link

Glucose Oxidase

Product code ISPT4. 3 separate 5mg antibody labellings.

£ 149.00

This provides an efficient method for labelling antibody with glucose-oxidase. The enzyme system can then be used for both ELISA and chemiluminescence methods.

#### Fluoro-Link

FITC

Product code FL-100 3 labellings of 1-10mg antibody.

£ 99.00

Product code FLM-200 3 labellings of 0.1 or 0.2mg.

£ 99.00

Fluorochromes are considered to be the most sensitive of direct visualisation techniques in immunohistological detection. The fluorescein isothiocyanate (FITC), provided, has an emission wavelength of 525nm (a strong green light). This gives good saturation of photographic materials for permanent records. Linked antibodies can also be used to target cells in applications such as fluorescence activated cell sorting.

#### Rhoda-Link

Rhodamine

Product code RL-100 3 labellings of 1-10mg antibody.

£ 99.00

Product code RLM-200 3 labellings of 0.1 or 0.2mg

£ 99.00

The kit labels antibodies with high coupling efficiency to rhodamine isothiocyanate, which has an emission wavelength of 570nm, giving a strong red light. Used in conjunction with FITC-labelled antibodies provides a simple method for double-staining of tissue sections or cells as a one-step, high sensitivity technique which can then be recorded by double-exposure photography.

#### Pep-Link kits:

Each kit provides sufficient material, including carrier proteins, for 2 completely independent conjugations of 10mg carrier protein to peptide, ample for most immunisation and screening preparations during monoclonal or polyclonal antibody production. Full instructions and wipe-clean, laminated quick-reference guide are included.

|                      |                           |         |
|----------------------|---------------------------|---------|
| Product code PCI-TGB | Bovine Thyroglobulin      | £199.00 |
| Product code PCI-KLH | Keyhole Limpet Hemocyanin | £199.00 |
| Product code PCI-BSA | Bovine Serum Albumin      | £199.00 |
| Product code PCI-OVB | Ovalbumin                 | £199.00 |

## Material Safety Data Sheet

Name: Dimethyl Sulphoxide

Chemical Description:  $C_2H_6SO$ , Mw 78.13, clear liquid, solid at  $<19^{\circ}C$ .

Hazards: Harmful if inhaled or swallowed or in contact with the skin. Readily absorbed through the skin and may cause sensitisation.

Handling protection: Wear protective clothing, gloves, glasses and face mask. Do not breath dust or swallow. Do not expose to skin and eyes. Avoid prolonged or repeated exposure

First Aid: Flush eyes and skin with large amounts of water. Remove contaminated clothing. If swallowed, rinse mouth and seek medical advice. If inhaled, remove to fresh air.

Handling/storage: Wear protective clothing, gloves, glasses and face mask. Use only in a chemical fume hood. Store in a cool dry place, keep tightly closed, away from ignition sources.

Avoid: Copper wool, potassium permanganate, trichloroacetic acid, acid halides and halide compounds, strong oxidising/reducing agents.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Absorb into inert material, place in bag or bottle for disposal. Wash up any residual.

Fire precautions: Water, dry powder foam. Wear contained breathing equipment. May emit toxic fumes.

Disposal: Approved disposal service. Mix with combustible solvent and burn in a properly equipped incinerator.

The above information is for guide-line purposes only and may not be fully comprehensive. All products should only be handled by trained personnel. Immune Systems and affiliated companies are not liable for any damage caused in any way by the above material.

## Material Safety Data Sheet

Name: Sodium Azide

Chemical Description:  $NaN_3$ , Mw 65.01, crystalline solid.

Hazards: Highly toxic. Fatal if inhaled or swallowed or absorbed through the skin. May cause genetic damage. May cause explosions. May give toxic gases.

Handling protection: Wear protective clothing, gloves, glasses and face mask. Do not breath dust or swallow. Do not expose to skin and eyes. Avoid prolonged or repeated exposure

First Aid: Flush eyes and skin with large amounts of water. Remove contaminated clothing. If swallowed, rinse mouth and seek medical advice. If inhaled, remove to fresh air.

Handling/storage: Wear protective clothing, gloves, glasses and face mask. Use only in a chemical fume hood. Store in a cool dry place. Keep tightly closed.

Avoid: Sodium azide may react with heavy metals and metal halides to form explosive products. Avoid acids. May explode when heated.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Place dry contents in bag or bottle for disposal. Wash up any residual.

Fire precautions: Dry powder only. Wear contained breathing equipment. May emit toxic fumes.

Disposal: Approved disposal service.

The above information is for guide-line purposes only and may not be fully comprehensive. All products should only be handled by trained personnel. Immune Systems and affiliated companies are not liable for any damage caused in any way by the above material.



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**LinKit™ Bio-Link (BL-100)**

**ISL**

## **Antibody & Protein Biotinylation**

### **Guide-Lines For Use**

(For full instructions refer to the manual)

- 1) Prepare 3ml purified antibody for labelling.
- 2) Set up one buffer exchange column and wash with 20ml biotinylation buffer.
- 3) Run 3ml of antibody solution onto the column, discarding eluate.
- 4) Place the reaction vial at the column outlet, add 4.5ml biotinylation buffer to the column and collect the eluate. Wash the column with 20ml storage buffer.
- 5) Dissolve one vial of biotinylation reagent in the required amount of solvent.
- 6) Slowly add the biotinylation reagent solution to the reaction vial containing the antibody to be labelled.
- 7) Stir for 4 hours at room temperature.
- 8) Make reaction volume to 6ml by addition of storage buffer. Add 3ml of the reaction mixture to the buffer exchange column, discarding eluate.
- 9) Place the collection vial at the column outlet. Add 4.5ml storage buffer to the column and collect the eluate.
- 10) Wash the column with 20ml storage buffer. Buffer exchange the remaining labelled antibody.
- 11) Store biotinylated antibody at 4°C in the dark.

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