LINKIT™

FLUORO-LINK

ANTIBODY & PROTEIN Fluoresceination

Instruction Protocol
1-10mg

PRODUCT CODE FL100

LinKit Fluoro-Link (Code FL-100)

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. Fluoresceination 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. Vials of solvent for fluorescein label, 3 x 1.25ml. Store at +4°C - +25°C. **HAZARDOUS,** avoid contact with skin and eyes or swallowing. In the event of contamination, seek immediate medical advice.

F. Vials of fluorescein label, 3 x 1mg. Store at -20°C. **HAZARDOUS,** avoid contact with skin and eyes or swallowing. In the event of contamination, seek immediate medical advice.

G. Reaction blocking buffer, 3 x 5ml. Store at +4°C - +25°C.

H. Collection tube, x 3. Store at +4°C - +25°C.

On receipt, the entire kit may be stored at +4 to +8°C, but do not freeze. Alternatively to save space in fridges, remove the 3 vials of Fluorescein label and store this at +4 to +8°C and the remaining kit components at room temperature.

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 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 approximately 10 - 12 hours to complete. First the antibody preparation is transferred to fluoresceination buffer. The fluorescein label is added and the reaction allowed to proceed for 6 - 8 hours at 4°C. The reaction is then blocked for 2 hours, or overnight at 4°C. The fluoresceinated antibody preparation is then transferred to phosphate buffered saline containing 0.1% sodium azide preservative, for storage and subsequent use

4. The labelling ratio obtained may be determined by measuring absorbance at 495nm (Fluorescein) and 280nm (antibody). A ratio of about 0.8 is adequate.

   The Fluorescein label is light sensitive. As an additional precaution against loss of activity bottles can be wrapped in aluminium foil during the reaction steps and blocking steps and for storage.

THE PROTOCOL

You are advised to wear safety glasses and gloves during and after use of the Fluorescein Label and Solvent.

A. Exchange to Fluoresceination Buffer

1. Unpack one filtration column **(A)**. Remove the upper cap and pour off the excess buffer. Place the column vertically in a rack or clamp stand, remove the outlet cap and allow any excess buffer to drain to waste.

2. Add 20ml of fluoresceination 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 fluoresceination buffer **(B)** to the column and collect the sample in the reaction tube.

5. Remove the reaction vial. Add 20ml storage buffer **(D)** to the reservoir of the column and allow to drain whilst continuing with the conjugation. Cap the outlet with the yellow closure supplied once the column has drained.

B. Fluorescein Labelling.

The following protocol gives an approximate molar ratio of 20 fluorescein to each antibody. However, as the reaction site for the fluorescein 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 fluorescein 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 gently warmed until melted.

2. Using a glass pipette, transfer 1ml of solvent to the vial containing the fluorescein label **(F)**. Re-cap the vial of label and stir briefly to dissolve the contents.

3. For each milligram of antibody in the sample to be labelled, add 30µl of dissolved fluorescein label, in small drops, to the antibody sample in the reaction vial **(C)** whilst gently stirring.

4. Re-cap the reaction tube and place in a 4°C fridge in the dark for 6 - 8 hours, stirring occasionally.

C. Blocking Reaction.

B. Add 300µl of blocking reagent **(G)** to the labelled antibody.

C. Stir briefly but thoroughly and stand at 4°C for 2 hours or overnight.

D. Exchange to Storage Buffer.

1. Make up the reaction volume to 6ml by addition of 1ml of storage buffer. Add half (ie 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 **(H)** at the column outlet to collect the fluorescein labelled antibody. Add 4.5ml storage buffer and collect the eluted sample in the collection tube. Place the collection tube in the dark or wrap in foil.

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.

7. Place the collection tube **(H)** at the column outlet to collect the fluorescein labelled antibody. Add 4.5ml storage buffer to the column and collect the eluted sample.

6. Store the fluoresceinated antibody preparation at 4°C in the dark.

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 fluorescein label added. However, it is important not to exceed the *volumes* indicated as this will give losses and inefficient buffer exchange.

Dialysis may be used at section D. 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 FITC linking ratio

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

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

Greater fluorescein to antibody labelling ratios can also be produced by addition of up to 600µl of fluorescein label at stage B3. The quantity of blocking solution required must also be increased, always adding an equal volume of blocking solution to the volume of fluorescein label added. To compensate, the quantity of storage buffer added prior to buffer exchange must be reduced to maintain a total volume of 6ml.

To increase the labelling ratio still further, dissolve the fluorescein label in a smaller quantity of solvent at step B2 above.

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

Other Antibody Labelling Kits

☐ Peroxi-Link — **Horseradish-Peroxidase**

Product code ISP7. 3 separate 5mg antibody labellings. £ 149.00

Horseradish 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 ISP8. 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 ISP14. 3 separate 5mg antibody labellings. £ 149.00

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

☐ Bio-Link — **Biotin**

Product code BL-100. 3 labellings of 1-10mg antibody. £ 99.00

Product code BLM-200. 3 labellings of 0.1 or 0.2mg. £ 99.00

A rapid method for labelling antibody with biotin, giving high labelling efficiency of approximately 15-20 biotin molecules/antibody molecule. Biotin is a versatile label in immunochemistry techniques, since a variety of detection reagents are available linked to avidin or streptavidin which bind specifically to biotin.

☐ 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 o 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 PC1-TGB	Bovine Thyroglobulin	£199.00
Product code PC1-KLH	Keyhole Limpet Hemocyanin	£199.00
Product code PC1-BSA	Bovine Serum Albumin	£199.00
Product code PC1-OVB	Ovalbumin	£199.00

Material Safety Data Sheet

Name: Dimethyl Sulphoxide

Chemical Description: C_2H_6SO, Mw 78.13, clear liquid, solid at <19°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.

Material Safety Data Sheet

Name: Fluorescein isothiocyanate

Description: Orange powder. Virtually insoluble in water.

Hazards: May be harmful by ingestion. May cause allergic reactions. Irritating to eyes.

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: Can react with oxidising materials.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Mix with sand and place dry contents in bag or bottle for disposal. Wash up any residual with water and detergent.

Fire precautions: Dry powder only. Wear contained breathing equipment.

Disposal: Approved disposal service.

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LinKit™Fluoro-Link (FL-100)

ISL

Antibody & Protein Fluoresceination

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 fluoresceination buffer.

3) Run 3ml of antibody solution onto the column, discarding eluate.

4) Place the reaction vial at the column outlet, add 4.5ml fluoresceination buffer to the column and collect the eluate. Wash the column with 20ml storage buffer.

5) Dissolve one vial of fluoresceination reagent in the required amount of solvent.

6) Slowly add the fluoresceination reagent solution to the reaction vial containing the antibody to be labelled.

7) Stir and stand for 6-8 hours at 4°C.

8) Add blocking reagent to the reaction vial and stand for 2 hours or overnight at 4°C.

9) Make reaction volume to 6ml by addition of storage buffer. Add 3ml of the reaction mixture to the buffer exchange column, discarding eluate.

10) Place the collection vial at the column outlet. Add 4.5ml storage buffer to the column and collect the eluate.

11) Wash the column with 20ml storage buffer. Buffer exchange the remaining labelled antibody.

12) Store fluoresceinated antibody at 4°C in the dark.

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