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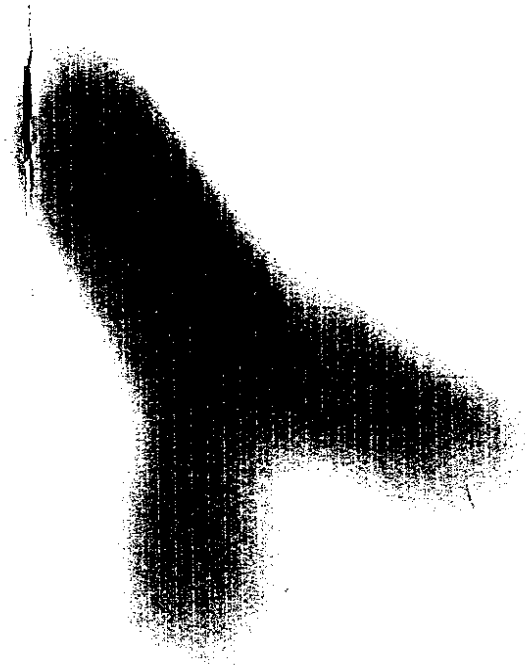

ISL

TM

LinKit

FLUORO-LINK

ANTIBODY & PROTEIN Fluoresceination

Instruction Protocol
100-200ug

PRODUCT CODE FLM200

LinKit Fluoro-Link (Code FLM200)

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 125ml. 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. Antibody samples for labelling should be at a concentration of 0.1mg/ml or 0.2mg/ml and free from stabilising proteins such as bovine serum albumin. Recovery will be in the region of 95%.

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 a additional precaution against loss of activity bottles can be wrapped in aluminium foil during the reaction 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 15ml 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 1ml 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 1.5ml fluoresceination buffer **(B)** to the column and collect the sample in the reaction tube.

5. Remove the reaction tube. Add 15ml 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.

For 0.1mg of antibody

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 by altering the volume of fluorescein label added at stage 2 below. To reduce the ratio, add a smaller volume of fluorescein label. Greater fluorescein to antibody labelling ratios can also be produced, if required, by addition of up to 200µl of fluorescein label at stage 2. This high conjugation ratio may lead to reduced antibody reactivity.

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 micropipette, transfer 0.5ml of solvent to the vial containing the fluorescein label **(F)**. Re-cap the vial of fluorescein label and stir briefly to dissolve the contents.

3 Return 50µl of dissolved fluorescein label to the vial of solvent **(E)** and mix thoroughly. Add 50µl of this diluted stock in drops to the antibody sample in the reaction tube **(C)** whilst gently shaking.

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.

1. If 50µl or less of fluorescein reagent was added at step 2 of the labelling stage above, add 50µl of blocking reagent **(G)** to the reaction tube **(C)** containing the labelled antibody.

   If more than 50µl of fluorescein reagent was added at step 2 of the labelling stage above, add an equal volume of blocking reagent **(G)** to the reaction tube **(C)** containing the labelled antibody. For example, if 125µl of fluorescein labelling reagent was added, now add 125µl of blocking reagent.

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

D. Exchange to Storage Buffer

1. If 50µl or less of fluorescein reagent was added at step 2 of the labelling stage above, make the reaction volume to 2ml by addition of 400µl of storage buffer.

   If more than 50µl of fluorescein reagent was added at step 2 of the labelling stage above, less storage buffer will have to be added. To determine the volume of storage buffer required, subtract **double** (to allow also for the extra blocking reagent added) the volume of fluorescein reagent added from 500µl.

2. Add 1ml (i.e. half) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

3. Place the collection tube **(H)** at the column outlet to collect the fluorescein labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample in the collection tube. Place the collection tube in the dark o wrap in foil.

4. Add 15ml storage buffer to the column and drain to waste.

5. Add 1ml (i.e. the remainder) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

6. Place the collection tube **(H)** at the column outlet to collect the fluorescein labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample.

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

For <u>0.2mg</u> of antibody

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, if required, by altering the volume of fluorescein label added at labelling step 2 below. To reduce the ratio, add a smaller volume of fluorescein label. Greater fluorescein to antibody labelling ratios can also be produced, if required, by addition of up to 200µl of fluorescein label at labelling step 2. This high conjugation ratio may lead to reduced antibody reactivity.

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 micropipette, transfer 0.3ml of solvent to the vial containing the fluorescein label **(F)**. Re-cap the vial of fluorescein label and stir briefly to dissolve the contents.

3 Return 60µl of dissolved fluorescein label to the vial of solvent **(E)** and mix thoroughly. Add 50µl of this diluted stock in drops to the antibody sample in the reaction tube 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.

1. If 50µl or less of fluorescein reagent was added at step 2 of the labelling stage above, add 100µl of blocking reagent **(G)** to the reaction tube **(C)** containing the labelled antibody.

   If more than 50µl of fluorescein reagent was added at step 2 of the labelling stage above, add 2x the volume of blocking reagent **(G)** to the reaction tube **(C)** containing the labelled antibody. For example, if 125µl of fluorescein labelling reagent was added, now add 250µl of blocking reagent.

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

D. Exchange to Storage Buffer

1. If 50µl or less of fluorescein reagent was added at step 2 of the labelling stage above, make the reaction volume to 2ml by addition of 350µl of storage buffer.

   If more than 50µl of fluorescein reagent was added at step 2 of the labelling stage above, less storage buffer will have to be added. To determine the volume of storage buffer required, subtract **3x** (to allow also for the extra blocking reagent added) the volume of fluorescein reagent added from 500µl (clearly for >165µl of fluorescein reagent, no storage buffer will be required).

2. Add half (i.e. 1ml in most cases, though where >165µl of fluorescein buffer was added the volume added to the column may be as much as 1.05ml) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

3. Place the collection tube **(H)** at the column outlet to collect the fluorescein labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample in the collection tube. Place the collection tube in the dark o wrap in foil.

4. Add 15ml storage buffer to the column and drain to waste.

5. Add the remainder of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

6. Place the collection tube **(H)** at the column outlet to collect the fluorescein labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample.

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

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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