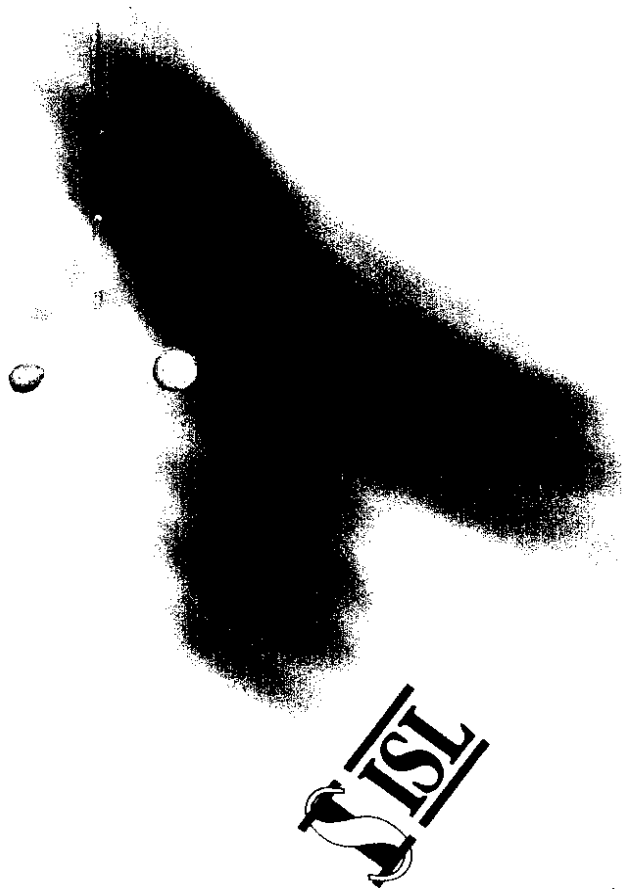
#1 SP 8

LinKit™



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LinKit™ Phospha-Link (Code ISP8)

Protocol Contents

A. Dialysis tubing x 3. Store at +4°C to + 20°C.

B. Water, 3 vials, Store at +4°C to + 20°C.

C. Reaction tube, 3 tubes containing magnetic stirrer bars and the alkaline phosphatase. Store at 4°C.

D. Activation Reagent for alkaline phosphatase, 3 x 1ml ampoules 25% Aqueous Glutaraldehyde, **Store at -20°C**.

This reagent is toxic and should only be handled in a fume cupboard. wear suitable protective clothing when handling this reagent, including gloves. For further information, call ISL.

E. Conjugation buffer, 3 tubes. Store at +4°C to + 20°C.

F. Gel filtration column, Store at +4°C to + 20°C.

G. 2M TRIS buffer, 3 vials, Store at +4°C to + 20°C.

H. Blocking reagent, 3 vials. Store at +4°C to + 20°C.

This reagents is extremely hazardous as it is both highly flammable and highly toxic. a copy of the original manufacturers data sheet is included in the manual, which should be read before proceeding. Wear suitable protective clothing when handling this reagent, including gloves and handle only in a fume cupboard. For further information, call ISL.

J. Storage buffer, contains 0.05% sodium azide as preservative, 6 pots. Store at +4°C to + 20°C.

K. Collection Tube, 3 tubes. Store at +4°C to + 20°C.

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 conjugations.

2. Antibody samples for conjugation should be prepared free from contaminating proteins at a concentration of 5 milligrams in 2.5ml.

3. The conjugation procedure takes 6 hours to complete. Firstly the alkaline phosphatase is activated. Secondly, the antibody preparation for conjugation is buffer exchanged into conjugation buffer. The antibody and alkaline phosphatase are then mixed for conjugation to occur. Finally remaining free activated groups are blocked and antibody conjugate is dialysed into storage buffer containing 0.05% sodium azide preservative.

   You may wish to store your antibody in a different buffer; this chosen buffer should be substituted for the Storage Buffer in the Protocol. It is recommended that 0.05% sodium azide or another suitable preservative is added to the conjugated antibody before storage at 4°C. However, the buffer supplied has balanced salts to optimise the stability of the conjugate.

The Protocol

You are advised to wear gloves throughout the conjugation process.

You will need the use of a fume cupboard, a magnetic stirrer and micropipettes in the range 10µl-1ml. In addition, approximately 1500ml double distilled wate is required to reconstitute the conjugation and storage buffers and a small quantity of ice is required to cool the reaction tube prior to and during the conjugation step.

A. Activation of the alkaline phosphatase.

Before beginning the conjugation, take 1 strip of dialysis tubing (A) and place this in a container with 1 litre distilled water at room temperature. To aid rehydration and washing, this may be brought to the boil and then allowed to stand.

1. Add 1.5ml water (B) to one reaction tube (C) containing the alkaline phosphatase. Swirl gently to dissolve contents. Thaw one ampoule of the activation reagent (D). Add 15µl of this to the reaction tube (C) in the fume cupboard. Stir gently for 50 minutes at room temperature.

B. Conjugation

1. Make up one vial of conjugation buffer (E) with 100ml distilled water, shake well to dissolve.

2. Unpack one filtration column (E). Remove the upper cap and pour off the excess buffer. Place the column vertically in a clamp stand and snap off the bottom tip allowing any residual buffer to drain to waste.

3. Add 20ml conjugation buffer (E) to the column and allow to drain to waste.

4. Add 2.5ml antibody sample to the column and allow the column to drain.

5. Place the column over the reaction tube in the fume cupboard. Add 3ml conjugation buffer to the column and collect the sample in the reaction tube.

6. Incubate for 75 minutes at room temperature with gentle stirring.

7. Wash the gel filtration column twice by filling the reservoir with residual conjugation buffer and draining to waste. The column can now be re-used immediately for the next conjugation or should be stored as outlined in section D1 below.

C. Blocking.

1. Stand the reaction vial in a small beaker of crushed ice on the magnetic stirrer.

2. Take one vial of 2M TRIS buffer (G) and whilst still cold, add 300µl to the reaction vial and continue to stir for a further 30 minutes. Discard the excess TRIS buffer.

3. Immediately prior to use, take one vial of blocking reagent (H) and stand alongside the reaction vial on the ice. Wearing gloves, add 1ml water (B) to the blocking reagent and shake briefly to dissolve.

4. Add 300µl of the dissolved blocking reagent to the reaction tube and stir for a further 2 hours 30 minutes. The mixture may froth slightly.

D. Exchange of Buffer.

1. Reconstitute the 1 pot of storage buffer (J) with 500ml of distilled water in a flask or measuring cylinder, this gives 0.1M sodium chloride, 0.1M magnesium chloride, 0.1 M Tris, 0.05% sodium azide.

   Fill the reservoir of the buffer exchange column with this buffer and allow to drain. Fit a yellow cap to the outlet, add a few ml's of storage buffer to the reservoir, fit the cap and store at +4°C to + 20°C.

2. Lift the dialysis tubing from the water and securely knot one end. Gently roll the other end between a thumb and forefinger to open the tubing.

3. Wearing gloves, gently pipette the contents of the conjugation tube into the open end of the dialysis tubing and allow the fluid to settle to the knotted end.

4. Securely knot the open end and place the sealed tubing in one flask of storage buffer and stand at room temperature or preferably 4°C for 24 hours with gentle stirring.

5. Reconstitute a second pot of storage buffer (J) with 500ml of distilled water in a flask or measuring cylinder. Transfer the dialysis tubing to the fresh storage buffer and stand at 4°C for a further 24 hours with occasional gentle stirring.

6. Lift the dialysis tubing from the storage buffer and allow the contents to settle to one end. Carefully cut off the knot at the top and pipette the contents into the storage vial provided. Store at 4°C.

Protocol Note

Storage.

The preparation obtained is a mixture of antibody-alkaline phosphatase complexes and unbound alkaline phosphatase and unbound antibody. This should not compromise the activity of the conjugated antibody.

If however you require separation of antibody-alkaline phosphatase complexes from unbound alkaline phosphatase and unbound antibody the mixture can be separated by gel chromatography. However, the blocking reagent liberates gas and when placed on gel columns will dry the column preventing separation. Consequently, dialysis must be carried out first.

10mg/ml bovine serum albumin may be added to the conjugated antibody to give additional stability.

Other Antibody Labelling Kits

☐ Peroxi-Link **Horseradish-Peroxidase**

Product code **ISP7.** 3 separate 5mg antibody labellings.

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.

☐ Gluco-Link **Glucose Oxidase**

Product code **ISP14.** 3 separate 5mg antibody labellings.

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

☐ Bio-Link **Biotin**

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

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

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.

☐ Fluoro-Link **FITC**

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

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

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.

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

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 **PC1-TGB**	Bovine Thyroglobulin
Product code **PC1-KLH**	Keyhole Limpet Hemocyanin
Product code **PC1-BSA**	Bovine Serum Albumin
Product code **PC1-OVB**	Ovalbumin

Material Safety Data Sheet

Name: Gltaraldehyde 25% aqueous

Chemical Description: Colourless liquid, strong odour.

Hazards: Harmful if inhaled or swallowed, irritating to eyes, may cause skin sensitisation.

Handling protection: Wear protective clothing, gloves, glasses and face mask. Do not 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: Avoid oxidisers.

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

Fire precautions: Dry powder, carbon dioxide, water or foam. Wear contained breathing equipment. May emit toxic fumes.

Disposal: Approved disposal service.

Material Safety Data Sheet

Name: Sodium Borohydride

Chemical Description: White solid.

Hazards: Highly toxic. Fatal if inhaled or swallowed or absorbed through the skin. May cause explosions. May give toxic gases. Can react explosively with dimethylformamide. Flammable on contact with water.

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: Water, acids, combustible materials and oxidising agents.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Mix with dry sand in a dry bag or container. Remove to an open space and slowly add a large quantity of water until the reaction is conplete.

Fire precautions: Dry powder or sand ONLY. Wear contained breathing equipment. May emit toxi 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.

Material Safety Data Sheet

Name: Sodium Azide

Chemical Description: NaN3, 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.

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