Material Safety Data Sheet

Name: Thimerosal

Chemical Description: $C_9H_9HgNaO_2S$ 0.01% solution when in use.

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: Avoid oxidising agents, strong acids, strong bases.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Mix with sand or other absorbent, 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.

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.

LinKit™ Peroxi-Link (Code ISP7)

Protocol Contents

A. **Storage buffer (10 x concentrate), x3.** Store at +4°C to + 20°C.
THIS REAGENT IS TOXIC. Read the MSDS included at the end of the manual before proceeding. wear suitable protective clothing when handling this reagent. For further information, call ISL.

B. **Activation tube, x3.** containing magnetic stirrer bars and the peroxidase.
Store at -20°C.

C. **Activation buffer, x3.** Store at +4°C to + 20°C.

D. **Activation reagent: Sodium-m-periodate, x3.** Store at +4°C to + 20°C.
STRONG OXIDISING AGENT, HANDLE WITH CARE. Read the MSDS included at the end of the manual before proceeding. wear suitable protective clothing when handling this reagent. For further information, call ISL.

E. **Coupling buffer, x2.** Store at +4°C to + 20°C.

F. **Buffer exchange column, x3.** Store at +4°C to + 20°C.

G. **Conjugation reaction tube, x3.** Store at +4°C to + 20°C.

H. **Stop buffer, x3.** This is sterile and excess should be discarded after the conjugation. Store at +4°C to + 20°C.

J. **Collection tube, x3.** Store at +4°C to + 20°C.

K. **Blocking reagent: Sodium borohydride, x3.** Store at +4°C to + 20°C.
THIS REAGENT IS EXTREMELY HAZARDOUS AS IT IS BOTH HIGHLY TOXIC AND EVOLVES FLAMMABLE GAS. Read the MSDS included at the end of the manual before proceeding. wear suitable protective clothing when handling this reagent., including gloves and handle only in a fume cupboard. For further information, call ISL.

L. **Dialysis tubing,** x3. Store at +4°C to + 20°C.

In addition you will require 900ml of deionised or double distilled water for each conjugation.

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.

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Introduction

1. The Protocol contains sufficient reagents for 3 antibody conjugations.

2. Antibody samples for conjugation should be prepared free from contaminating proteins. Any quantity of antibody in the range of 1 - 10mg is suitable for use in this protocol, but the starting volume of antibody should not exceed 1.5ml.

3. The conjugation procedure takes 5 -6 hours to complete with two incubations each of 2 hours. A 24 hour dialysis step is required at the final step.

4. Firstly the antibody is prepared for conjugation.

5. The horseradish peroxidase enzyme is activated and prepared for conjugation.

6. The antibody and enzyme are linked.

7. The reaction is halted and finally the antibody preparation is dialysed into storage buffer containing 0.01% Thimerosal preservative.

   You may wish to store your antibody in a different buffer which may be substituted for the storage buffer supplied with the kit. It is, however, recommended that sodium azide is **NOT** used as a preservative as this may affect the stability of the conjugates and the performance of the peroxidase (0.01% Thimerosal is recommended).

The Protocol

You are advised to wear gloves throughout the conjugation process. The reaction blocking stage of the process requires the use of a fume cupboard. In addition the use of a magnetic stirrer or mechanical roller and pipettes and micro-pipettes in the range 100µl to 5ml are required.

Before starting the conjugation, for each antibody to be conjugated, mix the contents of 1 bottle of **Storage buffer 10x concentrate (A)** into 900ml of deionised water in a flask or measuring cylinder (final volume = 1 litre).

A. <u>Preparation of Antibody.</u>

If the antibody for conjugation is available as a freeze dried preparation without salts or stabilisers, dissolve in 2.5ml of **coupling buffer (E)** and go to Section D: Conjugation.

For all other preparations follow the instructions below.

1. Unpack a **buffer exchange column (F)**. Remove the upper cap and pour off the buffer. Place the column vertically in a clamp stand, remove the bottom tip closure and allow the column to drain completely.

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 complete.

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

Disposal: Approved disposal service.

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Material Safety Data Sheet

Name :Sodium Periodate. (sodium-m-periodate).

Chemical Description: White crystalline solid.

Hazards: Harmful by ingestion and if inhaled as dust. Irritating to skin and eyes. Strong oxidising agent.

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 away from combustible materials. Keep tightly closed.

Avoid: Combustible materials.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Do not use combustible materials: paper etc. Mix with wet sand, place contents in bag or bottle for disposal. Wash up any residual.

Fire precautions: Water spray. Wear contained breathing equipment. May emit toxic fumes.

Disposal: Approved disposal service.

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2. Fill the upper reservoir with **coupling buffer (E)** and allow the column to drain to waste. Refill the column reservoir with **coupling buffer (E)** and drain to waste.

3. Make the antibody up to a volume of 1.5ml by adding **coupling buffer (E)**.

4. Add the antibody solution to the column and allow to drain to waste.

6. Place the **conjugation recation tube (G)** under the column outlet. Add 2.5ml **coupling buffer (E)** to the top of column and collect all the column effluent.

7. Wash the **buffer exchange column (F)** by filling the reservoir with **coupling buffer (E)** and allowing the column to drain to waste. Refill the column reservoir with **coupling buffer (E)** and drain to waste.

B. Activation of the Peroxidase.

1. Remove one **activation tube (B)** containing the peroxidase from the freezer and reconstitute with 1ml of **activation buffer (C)**.

2. Add 1ml of **activation buffer (C)** to one tube of **activation reagent (D)** containing the sodium meta-periodate.

3. Pipette 0.1ml of dissolved **activation reagent (D)** into the **activation tube (B)**.

4. Mix gently on a magnetic stirrer or mechanical roller for 20 minutes at room temperature (15-20°C).

C. Collection of the Activated Peroxidase.

1. After the 20 minute peroxidase activation time, add 0.4ml of **stop buffer (H)** to the activation tube containing the activated peroxidase. Pipette this solution into the reservoir of the washed buffer exchange column and allow to drain to waste.

2. Place the **collection tube (J)** under the column outlet. Add 2.5ml **coupling buffer (E)** to the top of column and collect all the column effluent.

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D. Conjugation.

1. For each milligram of antibody prepared for conjugation, pipette 225µl of peroxidase solution from the **collection tube (J)** into the **conjugation reaction tube (G)**.

2. Mix gently on a magnetic stirrer or mechanical roller for 2 hours at room temperature (15-20°C).

E. Blocking.

1. In a fume hood add 1ml of **activation buffer (C)** to one vial of **blocking reagent (K)** and swirl gently to dissolve.

2. Pipette 250µl of the **blocking reagent solution (K)** into the **conjugation reaction tube (G)** containing the enzyme-antibody mix. Swirl gently to mix. DO NOT INVERT.

   IMPORTANT SAFETY INFORMATION The blocking reaction will liberate hydrogen gas and the cap of the conjugation tube should be left loose as a precaution against pressure build up. Hydrogen gas is also flammable if confined so precautions against ignition should also be observed.

3. Place the **conjugation reaction tube (G)** at 4-8°C for a maximum of 2 hours, swirling the contents occasionally.

F. Dialysis into Storage Buffer.

1. Whilst the blocking reaction takes place, take 1 strip of **dialysis tubing (L)** for each antibody being conjugated and place these in a container with 500ml distilled water at room temperature. To aid re-hydration and washing, this may be brought to the boil and then allowed to stand.

2. After the 2 hour blocking step, wearing gloves, lift the **dialysis tubing (L)** from the water and securely knot one end. Gently roll the other end between a thumb and forefinger to open the tubing.

3. Carefully pipette the enzyme-antibody mix from the **conjugation reaction tube (G)** into the **dialysis tubing (L)** and allow the fluid to settle to the knotted end.

4. Securely knot the open end and return the sealed tubing to the flask containing the diluted **storage buffer (A)** and stand at 4°C for at least 24 hours with occasional gentle stirring.

5. After dialysis lift the tubing from the storage buffer (A) and allow the contents to settle to one end. Carefully cut off the knot at the top and aliquot the contents into a suitable tube or multiple tubes for storage. Store at 4°C.

Protocol Notes

Peroxidase is extremely sensitive to azide and thimerosal is the preferred choice of preservative in all buffers.

If a precipitate forms this may be removed by centrifugation.

The mixture may be further purified by separation of antibody-peroxidase complexes from unbound peroxidase and unbound antibody by centrifugation and gel chromatography. However, as the blocking reagent liberates gas, dialysis must be carried out first.

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

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