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<tbody>
<tr>
<td>KT20</td>
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</tr>
<tr>
<td>KT20A</td>
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<td>KT20B</td>
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Revision No.: 00280205
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Objective:
To learn the technique of immunoelectrophoresis.

Principle:
Immunoelectrophoresis is a powerful technique to characterize antibodies. The technique is based on the principles of electrophoresis of antigens and immunodiffusion of the electrophoresed antigens with a polyspecific antiserum to form precipitin bands.

Electrophoresis:
During electrophoresis, molecules placed in an electric field acquire a charge and move towards appropriate electrode. Mobility of the molecule is dependent on a number of factors:

- It is proportional to field strength and net charge of molecule.
- Inversely proportional to frictional coefficient of the molecule which is dependent on size/shape of the molecule and viscosity of the medium.
- Heat generated by high ionic strength buffers.
- Changes in pH of buffer due to electrolysis of water.
- Endosmosis: The agarose matrix adsorbs hydroxyl ions on the surface during electrophoresis, resulting in a net increase in positive ions, which migrate towards the negative electrode with a solvent shell. This net solvent flow is referred to as endosmosis. Sample molecules migrating against these ions meet resistance and are hindered in their movement, whereas sample molecules migrating along with the ions move faster.
Thus, when antigens are subjected to electrophoresis in an agarose gel, they get separated according to their acquired charge, size and shape, by migrating to different positions.

**Immunodiffusion:**

Antigens thus resolved by electrophoresis are subjected to immunodiffusion with antiserum added in a trough cut in the agarose gel. Due to diffusion, density gradient of antigen and antibody are formed and at the zone of equivalence, antigen-antibody complex precipitates to form an opaque arc shaped line in the gel. The precipitin line indicates the presence of antibody, specific to the antigen. If the antibody is homogeneous only one precipitin line is visible. Presence of more than one precipitin line establishes the heterogeneity of antibody, while the absence of precipitin line indicates that the antiserum does not have antibody to any of the antigens separated by electrophoresis.

**Kit Description:**

In this kit, two antisera, one raised against a IgG and the other raised against whole serum are supplied. Antiserum A has antibodies against whole serum and hence contains a mixture of antibodies against serum proteins. It will thus form more than one precipitin line indicating the heterogeneity of the antisera. Antiserum B, has antibody against IgG. Since it contains a single antibody, only one precipitin line will be formed, indicating the specificity of the antibody against the antigen. Following immunoelectrophoresis, students will be able to establish the homogeneity/heterogeneity of the antisera.

- **KT20**: The kit is designed to carry out 5 immunoelectrophoresis experiments. The kit also includes immunoelectrophoresis equipment with accessories (ETS-2) required for immunoelectrophoresis.

- **KT20A**: The kit is designed to carry out 5 immunoelectrophoresis experiments.

- **KT20B**: The kit is designed to carry out 10 immunoelectrophoresis experiments. Two each of antiserum A and antiserum B vials are supplied, one vial each for 5 experiments.

**Note**: Immunoelectrophoresis equipment with accessories is required for KT20A and KT20B.
**Duration of experiment:** Experiment is carried out over a span of 2 days, approximate time taken on each day is indicated below:

Day 1: 3 hours 30 minutes (Electrophoresis and Immunodiffusion)
Day 2: 30 minutes (Observation and Interpretation)

**Materials Provided:**

The list below provides information about the materials supplied in the kit. The products should be stored as suggested. Use the kit within 6 months of arrival.

<table>
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<tr>
<th>Materials</th>
<th>Quantity</th>
<th>KT20/20A (5 expts.)</th>
<th>KT20B (10 expts.)</th>
<th>Store</th>
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<tbody>
<tr>
<td>Agarose</td>
<td>1.0 g</td>
<td>2.0 g</td>
<td>4°C</td>
<td></td>
</tr>
<tr>
<td>5X Electrophoresis buffer</td>
<td>200 ml</td>
<td>2 x 200 ml</td>
<td>4°C</td>
<td></td>
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<tr>
<td>Antigen</td>
<td>0.1 ml</td>
<td>0.2 ml</td>
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<tr>
<td>Test Antiserum-A</td>
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<td>2 x 1.5 ml</td>
<td>4°C</td>
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<td>Test Antiserum-B</td>
<td>1.5 ml</td>
<td>2 x 1.5 ml</td>
<td>4°C</td>
<td></td>
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</table>

**Materials Required:**

Glassware: Conical flask, Measuring cylinder.
Reagent: Distilled water.
Other Requirements: Micropipette, Tips, Moist chamber (box with wet cotton)
Note:

- Read the entire procedure before starting the experiment.
- Reconstitute the antiserum vials (A and B) by adding 1.5 ml of distilled water, store at 4°C and use within 3 months.
- In case of KT20B, reconstitute one set of antiserum vials for 5 experiments.
- Dilute required amount of buffer to 1X concentration with distilled water.

Procedure:

Preparation of gel plate

1. Prepare 10 ml of 1.5% agarose (0.15 g/10 ml) in 1X electrophoresis buffer by heating slowly till agarose dissolves completely. Take care not to scorch/froth the solution.
2. Mark the end of a glass plate that will be towards negative electrode during electrophoresis.
3. Place the glass plate on a horizontal surface. Pipette and spread 10 ml of agarose solution onto the plate. Take care that the plate is not disturbed and allow the gel to solidify.
4. Place the glass plate on the template holder provided in ETS-2 and fix the template. Punch a 3 mm well with the gel puncher as shown in the figure 1, towards the negative end.
5. Cut two troughs with the gel cutter provided (in ETS-2), but do not remove the gel from the trough.

-ve                         +ve

Fig.1: Pattern of addition of antigen and antisera to well and troughs respectively.

Electrophoresis

6. Add 12-15 µl of antigen to the well.
7. Place the glass plate in the electrophoresis tank such that the antigen well is at the cathode/negative electrode. Pour 1X electrophoresis buffer such that it covers the gel.
8. Set the voltage to 50-100V and electrophorese until the blue dye travels 3-4 cms from the well. Do not electrophorese beyond 3 hours, as it is likely to generate heat.

Immunodiffusion

9. Remove gel from both the troughs and keep the plate at room temperature for 15 min. Add 250 µl of antiserum-A in one of the troughs and antiserum B in the other.
10. Place the plate in a moist chamber and allow diffusion to occur at room temperature, overnight.
Observation:
Observe for precipitin lines between antiserum troughs and the antigen well. (Refer fig 2).

Interpretation:
- Presence/absence of precipitin line indicates the presence/absence of antibody specific to the antigen, respectively.
- Presence of more than one line indicates the heterogeneity of the antiserum to the antigen.
- Presence of a single precipitin line indicates homogeneity of the antiserum to the antigen.

Fig 2: Glass plate showing precipitin lines following immunoelectrophoresis.

Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat #</th>
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<tr>
<td>GeNei™ Immunoelectrophoresis (IEP) 1 Pack KT20 Teaching Kit (Consumables for 5 experiments &amp; Elpho Kit (ETS-2))</td>
<td>KT20</td>
<td></td>
</tr>
<tr>
<td>GeNei™ Immunoelectrophoresis (IEP) 1 Pack KT20A Teaching Kit (Consumables for 5 experiments)</td>
<td>KT20A</td>
<td></td>
</tr>
<tr>
<td>GeNei™ Immunoelectrophoresis (IEP) 1 Pack KT20B Teaching Kit (Consumables 10 experiments)</td>
<td>KT20B</td>
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</tbody>
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