

**GeNei™ Counter Current
Immunoelectrophoresis
Teaching Kit
Manual**

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KT29A	106164
KT29B	106166
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Objective:

To check antisera for the presence of antibody towards a specific antigen by Counter current immunoelectrophoresis (CCIEP).

Principle:

CCIEP is a rapid version of Ouchterlony double diffusion (ODD) technique which can be performed within an hour. It is primarily a qualitative test, although from the thickness of the precipitin line relative measure of quantity can be obtained.

Here the antigen is placed in a well at the cathode end and antibody is placed at the anode side. During electrophoresis molecules placed in an electric field acquire a charge depending on their pI (Isoelectric point). Hence they move towards the appropriate electrode. The antigen, if it is negatively charged moves towards the anode.

Antibody (Immunoglobulin) at pH 7.6 has a charge nearing zero. During electrophoresis, the agarose matrix absorbs OH⁻ ions on the surface resulting in a net increase in positive ions at a distant from the matrix. These positive ions migrate towards the negative pole with a solvent shield, resulting in a net solvent flow called endosmosis. Hence antibody molecules which have no charge move towards cathode along with solvent shield due to this phenomenon.

Thus the antigen and antibody travel towards each other and at a point where there is optimum concentration of both a line of precipitation (band) is formed.

Kit Description:

Using this kit, students will test for the presence of antibody in 3 different test antisera samples. The antigen supplied is BSA, which has negative charge at pH 7.6. BSA will be loaded into wells near **cathode** and antisera (positive and 3 test samples) towards **anode**. On electrophoresis, antigen will travel towards anode and the antibody towards cathode. At the equivalence point, a precipitin line will be formed in those antisera samples which have antibody towards BSA.

KT29 : The kit is designed to carry out 10 experiments. The kit also includes CCIEP equipment with accessories (ETS-2) required for the experiments.

KT29A : The kit is designed to carry out 10 experiments.

KT29B : The kit is designed to carry out 20 experiments.

Duration of experiment: Approximately 1 hour.

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.

Materials	Quantity		Store
	KT29/29A (10 expts.)	KT29B (20 expts.)	
Agarose	2 g	3 g	4°C
Antigen	0.5 ml	1 ml	-20°C
Test Antiserum	3 x 0.2 ml	3 x 0.3 ml	-20°C
Positive Control Antiserum	0.2 ml	0.3 ml	-20°C
Electrophoresis Buffer (5X)	3 x 125 ml	3 x 250 ml	4°C

Materials Required:

Glassware : Conical flask, Measuring cylinder.

Reagent : Distilled water.

Other Requirements: Tips, Micropipette.

Note:

- **Read the entire procedure before starting the experiment.**
- Reconstitute the antigen vial by adding 0.5 ml of distilled water, in case of KT29/KT29A and 1 ml in case KT29B. Store the sample at 4°C. Use within 3 months.
- Reconstitute the antiserum vial by adding 0.2 ml of distilled water in case of KT29/KT29A and 0.3 ml in case of KT29B. Store the sample at 4°C. Use within 3 months. Reconstitute all the vials, only if all 20 experiments will be carried out within 3 months.
- Dilute required amount of 5X Electrophoresis buffer to 1X concentration with distilled water before use.
- Wipe the glass plate with alcohol thoroughly to make it grease free for even spreading of agarose.
- Mark the end of the slide that will be towards positive electrode during the electrophoresis. Ensure that the slide is properly placed.

Procedure:

1. Prepare 10 ml of 1.5% agarose (0.15g/10ml) in 1X electrophoresis buffer by adding agarose to the buffer and heating slowly to dissolve the agarose completely.
2. Mark the end of the slide that will be towards positive electrode during electrophoresis.
3. Place the slide on a leveled tabletop and quickly pipette 7 ml agarose onto 50X75mm slide, spreading while releasing the agarose. Allow to solidify for 15 minutes.

Note: Do not disturb the slide.

4. Place the gel plate on the template holder provided (ETS-2) and fix the template for CCIEP. Punch 3 mm wells with the gel puncher corresponding to the markings on the template. (Refer Fig 1).

Note : Punch four pairs of wells for the experiment.

5. Place the slide in the electrophoresis tank and fill the tank with 1X electrophoresis buffer till the buffer just covers the gel surface. Do not add excess of buffer.

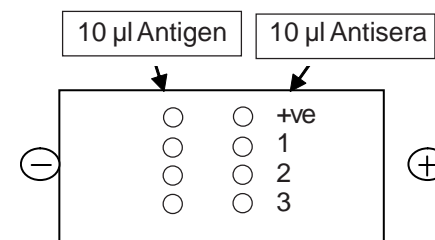


Fig 1: Pattern of addition of antigen and antiserum to the wells.

6. Add 10 μ l of antigen in each of the four wells towards cathode (Negative electrode) and 10 μ l of positive control antiserum and three test antisera in wells towards anode (Positive electrode) as shown in the Figure 1.
7. Connect the power cord to the electrophoretic power supply according to the convention:
red : anode and black : cathode.
8. Apply 50 V and allow the electrophoresis to continue for about 45 minutes.
9. Observe for precipitin line between the antigen and antisera wells.

Observation:

Note down the presence/absence of precipitin line between antigen and antisera wells as follows.

Sample	Precipitin Line
Control	
Test antisera 1	
Test antisera 2	
Test antisera 3	

+: Presence of precipitin line

-: Absence of precipitin line

Interpretation:

- a). Precipitin line indicates the presence of antibody for the antigen in the test sera.
- b). The absence of the precipitin line indicates the absence of any antibody for the antigen in the test sera.

Ordering Information

Product	Size	Cat #
GeNei™ Counter Current Immunoelectrophoresis Teaching Kit (Consumables 10 experiments & Elpho Kit (ETS 2))	1 Pack	KT29
GeNei™ Counter Current Immunoelectrophoresis Teaching Kit (Consumables 10 experiments)	1 Pack	KT29A
GeNei™ Counter Current Immunoelectrophoresis Teaching Kit (Consumables 20 experiments)	1 Pack	KT29B

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