

Double immunodiffusion

For classroom teaching

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Introduction

Diffusions reactions in gels are a range of methods of determining antigens or antibodies. The method was developed by the Swede Örjan Ouchterlony for about 30 years ago. His method is called "double" referring to the fact that in this procedure antigen and antibody are allowed to migrate towards each other in a gel and a line of precipitation is formed where the two reactants meet. This precipitation reaction is highly specific. The method is even today widespread and used by people working with diagnoses or protein detection or comparing antigens or antisera. The method is not very sensitive but very useful when you have enough of antigen or antibody.

Aim

To analyse solutions of antigens and antibodies by using double immunodiffusion.

Practical

45 minutes for casting the gel and loading the samples
20 minutes the following day or later for analysing the result.

Teachers note:

Safety

It is not necessary to use any protection.
But be aware that the gel solution can be very hot
And remember to teach the students to wash their hands etc.

Waste

Everything can be handled as normal garbage.

Punch pattern:

It may be necessary with some routine to punch a regular pattern in the gel. Therefore it can be an idea to start in the edge making the first punch pattern leaving space for a second trial.

Use a straw for punching (diameter 3 mm) and remove the little plug by sucking with the straw after putting a little gaze on the end, or remove the plug by using a needle.

After the first day:

If it is very warm or dry in the lab, the plates can be covered by a moist towel and plastic foil until the following day.

If it is necessary to keep the plates for many days it is recommended to keep the plates in a plastic bag (moist) with a little preservative to avoid mould.

The plates will be ready after one night or you can keep them for up to one week in the fridge.

Materials and chemicals (for one class)

Electrophoresis buffer (EDTA + Tris/tricine)

pH 6, 5x concentrated, 0.126M Tricin, 0.366M Tris, 10mM EDTA:

Dissolve 44.3 g Tris plus 22.6 g tricine in 500mL's of deionised water

Add 50 mL's of 0.2mL EDTA

Add water until a total volume of 1000mL

To be stored in the fridge

Agarose

Microwave oven or heating system

Antibody

Antigen

20 small Petri dishes (diameter 5 cm's) - plastic

20 small straws

20 pushpins

Pipettes for filling the wells

WHAT TO DO...

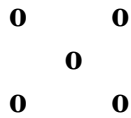
Casting the gel

1. Dissolve the right amount of agarose in
Electrophoreses buffer (10 g/L)
Use a microwave oven.
TAKE CARE!! IT "OVERBOILS" Easily
The solution should be clear when it is ready
2. Let it cool to 60-80 °C
3. Place the Petri dishes on a horizontal table close to the edge.
Remove the lids.
4. Pour the hot gel into the dishes - just covering the bottom is enough.
5. Let the gels set - will last about 5 minutes.
Put on the lids while not working with the dishes.

Punching the wells

6. Use the straw to punch the wells in the gel. Be careful and cut with vertical sides.

Remove the gel-plugs with the pushpin.
 Make the following well pattern:



Adding antibody:

Before filling the wells you should mark each well on the bottom of the Petri dish - so you can remember where to do what.

- 7. Fill the middle well with antibody. Usually 5-7 μL will be enough.
 Don't overfill – it will disturb the result

Adding antigen:

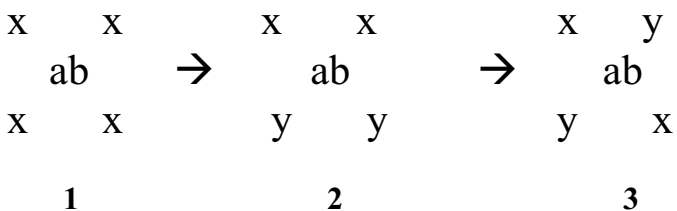
- 8. Fill the outer four wells with antigen/s
- 9. Close the dishes
 Leave them until the following day in a humid chamber at room temperature for incubating.

Results:

- 10. Study the plate. Make a drawing of what you can see.
 It's easier to see the precipitation lines if holding the Petri dish against a dark background.

Ideas:

This is an idea to a working procedure in a class.
 Let's suppose we are going to investigate two antigens - x and y.
 The antibody-solution placed in the centre-well contain two different antibodies - one against x and one against y.
 Group the students into 3, and let the students add antigens after the following pattern:



Group 1

They have the same antigen in all four wells, so

the 4 possible lines will melt together.

Group 2

The two upper wells have the same antigen and will melt together. The same can be said about the two lower wells.

The two lines cross since they are not identical.

Group 3

No identity with the antigens in the neighbour-well
- all lines cross each other.

Since the students don't have that much routine, the pattern may vary from the shown.

Let the students try to make other patterns.