

SINGLE IMMUNO DIFFUSION – RADIAL IMMUNO DIFFUSION

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Purpose

Using this relatively simple technique it's possible to make a simple qualitative investigation of antigens and antibodies. Antibodies as well as antigen(s) will diffuse through the gel and when reaching the equivalence point they will precipitate. As so rings will form around the centre. The size of the rings will be equivalent to the amount of antibodies.

Lessons

1 lesson (45 minutes) for preparing the plates and loading the samples
1 lesson for staining and interpretation

Safety

No special rules

Materials and chemicals

Precoated object glasses
2% agarose in Tris/tricine buffer
Antibody – e.g. anti-swine serum
Antigens like swine serum (IgG) in different concentrations 10, 50, 100, 150 and 200 μL pr 1mL
Unknown antigen
Phosphate buffer PBS
Straws or the like to punch holes in the gel
Humid chamber
Pasteur pipettes

WHAT TO DO...

1. Melt the agarose by using a microwave oven
Place the melted agarose in a water bath 56°C
2. Dilute the antibody – use $75\mu\text{L}$ to 1,9 mL PBS
Place the solution in the water bath
3. Take 1 mL of the melted agarose and pour it into the antibody. Mix well.
4. Carefully pour the mixture on a precoated plate. Let it solidify
5. Using a straw punch 5 holes into the gel The diameter of the holes should be 2-3 mm's.
The small gel-pieces can be removed by sucking with a pipette.
6. Add the different antigen-solutions into the wells. 10 μL in each well
7. Place the gel in a humid chamber to avoid drying. Leave until the next day

The following day:

8. Measure the diameter of the precipitation rings.
Use a dark background and light from the side to see the rings
9. Place the plate in a staining solution for 5 minutes
10. Destain by using ethanol 70% for 10 seconds-
The rings might be clearer and easier to measure.