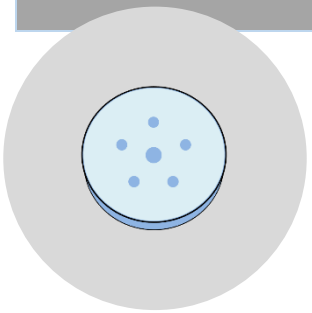


Ouchterlony double immunodiffusion

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Ouchterlony double immunodiffusion is a classical immunological technique used to detect the presence of specific antigens and antibodies in a sample. It is a simple and widely employed method that allows the qualitative analysis of antigen-antibody reactions by visualizing the formation of a precipitin line.

The principle behind Ouchterlony double immunodiffusion lies in the diffusion of antigens and antibodies through an agarose gel matrix. The diffusion allows the antigens and antibodies to encounter each other, leading to the formation of immune complexes if a specific antigen-antibody interaction occurs. These immune complexes precipitate out of solution, forming visible lines or bands at the point of interaction.

While newer techniques have emerged for quantitative analysis, this classic method continues to find applications in research, diagnosis, and antibody production, contributing to the understanding of the immune system and its responses.

Material		
Ouchterlony Plates	1x TAE 1.2 % Agarose (1.2 g / 100 ml)	
Antigen of interest		
Antibody of interest		
10x TAE	400 mM Tris-HCl 10 mM EDTA 200 mM Acetic Acid	In some cases, TAE-Buffer does not work. You may want to use Boric Acid/NaOH buffer instead.
Gel borer		

- 2 - Method

Gel Preparation

- Prepare 1.2 % agarose solution in 1 x TAE buffer
- Heat the solution until the agarose dissolves completely
- Allow the solution to cool down slightly
- Pour the agarose solution into a clean petri dish
- Allow the gel to solidify for approximately 20 minutes

Well Preparation

- Cut out a center well with a diameter of 8 mm
- Punch out multiple outer wells with a diameter of 6 mm
  The number and arrangement of the wells will depend on the number of samples being tested

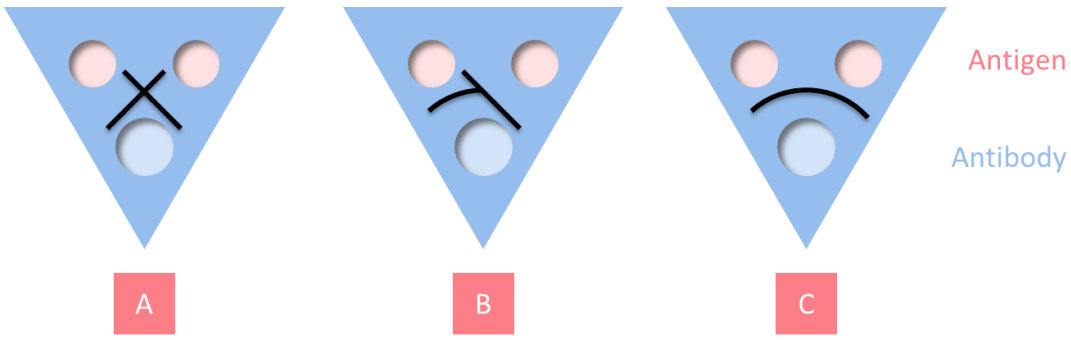
Sample Diffusion

- Add up to 50 µl of the desired antibody solution (1 – 10 mg/ml) to the central well
- Add up to 50 µl of the desired antigen solutions (0.1 – 1 mg/ml) to the outer wells
- Incubate the Ouchterlony plate at room temperature for 60 minutes
  Please note that the first precipitations and spur lines may appear after 20 minutes

Interpretation

The appearance and characteristics of the precipitin lines provide information about the specific interactions between antigens and antibodies. Factors such as the size and shape of the lines, their position, and the presence or absence of a spur can be analyzed.

- Non-Identity (A): Two spurs forming at the intersection of the precipitation lines indicate no match or non-identity between antigens and antiserum
- Partial Identity (B): One or more spurs branching out from the main precipitin line at the intersections suggest a partial match between antigens and antiserum
- Full Identity (C): Continuous precipitation line without a spur formation indicates a complete match between antigens in the outer wells and the antiserum in the central well



- 3 - Trouble Shooting

Reason	Solution
Well Punching	Using toothpicks to lift the cut agarose plugs from the Ouchterlony plates works well. Using the end of a glass Pasteur pipet as an alternative to a gel borer. Using a printed diagram outlining the Ouchterlony plate with markings for the wells as a guide.
Assay Controls	Using deionized water as a negative control, resulting in no precipitin lines. Using a positive control well with a known antigen-antibody interaction, resulting in a strong precipitin line.
No Precipitin Line Formation	Ensure that the antigens and antibodies used are present in high concentrations. Titrate the concentrations to optimize the reactions. Allow enough time for diffusion to occur. Increase the diffusion time or decrease the agarose concentration of the gel. Ensure the agarose gel to properly solidify before initiating the diffusion.
Satellite Lines	Ensure that all reagents and plates used are clean and free from contamination.
Cross-Reactivity	Use purified antigens and antibodies to minimize cross-reactivity. Consider performing additional tests to confirm the specificity of the reaction, such as inhibition assays.
Merging of Precipitin Lines	Optimize the concentrations of the samples, as excessive concentration of samples can lead to merging or spreading of precipitin lines.
Inconsistent Results	Ensure accurate and consistent dilution of samples. Maintain a controlled environment during the diffusion to minimize variability, caused by fluctuations in temperature and humidity. Maintain proper aseptic techniques throughout the experiment.