INSTRUCTIONS

Immunodiffusion Plates

31111 31113

0268.2

Number	Description
31111	**Immunodiffusion Plates, Agarose Gelling Agent, Multiple Pattern,** 6 each, contains 0.1% sodium azide as a preservative Well Diameter: 3.2mm Interwell Distance: 3.2mm Gel Depth: 2.8mm Well Volume: 22µL
31113	**Immunodiffusion Plates, Agarose Gelling Agent, Single Pattern,** 10 each, contains 0.1% sodium azide as a preservative Well Diameter: 5.0mm Interwell Distance: 4mm Gel Depth: 1.6mm Well Volume: 31µL

Storage: Upon receipt store at 4°C. Plates are shipped at ambient temperature. Do not freeze plates or allow them to become dry.

Introduction

The Thermo Scientific Immunodiffusion (ID) Plates are used for immunodiffusion, which is a technique for detecting antigen-antibody interactions based on the theory of double diffusion originally described by Oudin[1] and Ouchterlony.[2] Antigens and antibodies are placed into separate wells that are cut into a gel matrix and allowed to diffuse towards each other. If the reaction is positive, a precipitate forms that appears as an opaque line. The precipitation reaction occurs when the antigen and antibody concentrations are combined at near equivalent proportions.

Extensive binding must occur between the antigen and antibody for immune complexes to precipitate within the gel matrix. A divalent antibody displays two reactive sites capable of binding the antigen, and the antigen may contain numerous sites where the antibody can bind; however, generally molecules with fewer than four binding sites do not function well in immunodiffusion tests. Furthermore, IgM molecules are generally too large to diffuse in ID gels.

When multivalent antigens combine with divalent antibodies in solution, three-dimensional lattices are formed that aggregate and precipitate. The amount of precipitate varies in proportion to the concentration of the antigen and antibody. At equivalent or optimal proportions, almost all of the antigen and antibody will precipitate. If there is an excess of antibody, the complexes formed with the antigen are insoluble. When there is an excess of antigen, the precipitate has a tendency to dissolve caused by the formation of soluble complexes.

Additional Materials Required

- Moist Chamber: Any container that has a tight fitting cover, such as a petri dish, plastic box or glass jar, containing moist filter paper or toweling. The plates must be stationary and level during incubation.
- Reading Light: Use any light source that produces a beam of high-intensity light. For example, a penlight or a dissecting microscope light.

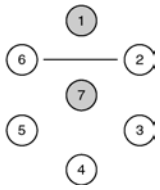
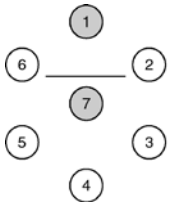
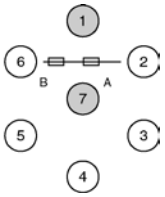
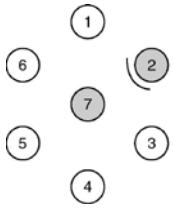
Example Procedure

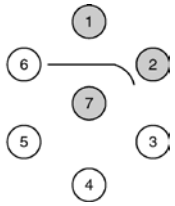
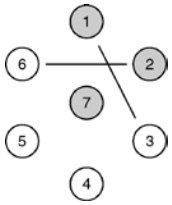
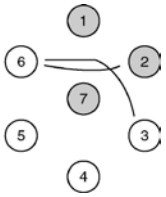
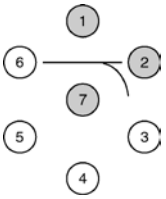
Note: Wells must be completely filled (i.e., 22µL and 31µL for Product No. 31111 and 31113, respectively). A known reactivity control should be used adjacent to any unknown samples for comparison and to optimize assay sensitivity (see the Example Tests and Possible Results Section).

1. Label the ID plate and place it on a dark surface for well filling. Record the plate identification and the well contents.
2. Use a pipette to add the antibody samples to the appropriate wells.

   Example: Place control sera in wells #1 and #4, reference antigen in well #7 and test samples (antigens or antibodies) in wells #2, #3, #5 and #6.
3. Because antibodies are generally larger than antigens and diffuse more slowly, place the plate in a moist chamber for 30 minutes before adding the antigen, which helps to center the ID bands.
4. Add the antigen into the appropriate wells.
5. Place the ID plate in a moist chamber on a level surface and incubate at room temperature for 24 hours. Highly dilute antigen and antibody solutions may require longer incubation.
6. To view the formed precipitin bands, use a beam of high-intensity light with the plate held over a dark background and the light projecting from below at approximately 45° angle to the plate surface.
7. Draw a diagram of the precipitin lines and record the number of lines observed.

Example Tests and Possible Results

Example 1: An antibody is placed in well #1 and its corresponding antigen is placed into the center well (well #7). After the diffusion occurs, a precipitin or immunodiffusion line forms in the region of the gel where an equivalence ratio occurs.	
Example 2: An antigen is added to well #7 and a high concentration of antibody is added to well #1. The resulting precipitin line is closer to well #7 because the high concentration of the antibody causes it to diffuse faster than the antigen.	
Example 3: Portions of the gel that contain the precipitated antigen-antibody complexes are removed and analyzed for concentrations. The concentration of the complexes is less at position B than at position A because position B is farther away from the wells. However, the ratio of the two reactants is the same at both positions because the precipitin forms where optimal interaction of antibody and antigen occurs.	
Example 4: An antigen is placed in well #7 and a low concentration of antibody is placed in well #2. The precipitin line is close to well #2 because of the low relative concentration of antibody. If the antibody concentration is too low, the equivalence zone can occur inside of well #2, giving the appearance of a negative result.	

Example 5: An antigen is added to well #7 and its corresponding antibody is added to well #1. A low concentration of antibody is added to well #2. Evaluating the end of the precipitin line, which is turned away from well #2, allows determination of low antibody concentrations.	
Example 6: To determine immunologic specificity, a mixture of two different antigens is added to well #7. The respective antibody samples are added to well #1 and well #2. After incubation, an intersection where the lines meet implies that the reactions have unrelated specificities, which are known as nonidentity reactions.	
Example 7: Similar to Example 6, two different antigens are added to well #7. An antibody sample is added to well #2, and a mixture of two antibodies is added to well #1. The result is a continuous arc between wells #1 and #7 and between wells #2 and #7. This is known as the identity arc, which indicates that the reactants in well #1 and well #7 and in wells #2 and #7 have the same immunological specificity. Also note that the arc does not affect the second band between wells #1 and #7.	
Example 8: Similar to Example 7, the relative concentrations of two different antibodies used in well #1 are such that the resulting bands are superimposed and indistinguishable. The antibody in well #2 forms an arc with the antigen in well #7, but a spur projects from the arc. This result is known as a partial identity and indicates that the specificities in the reactants in wells #1 and #7 are contained in the precipitin line between wells #2 and #7; however, the exact number of specificities in a single band cannot be determined.	

Cited References

1. Oudin, J.; L'analyse, (1948). immunochemique qualitative. Methode par diffusion des antigens au sein de l'immunoserum precipitant gelose. *Premiere Parte Inst Pasteur* **75:**30-52.
2. Ouchterlony, O. (1949). Antigen-antibody reactions in gels and the practical applications of this phenomenon in laboratory diagnosis of diphtheria. *Med Diss Stockholm.*