

Modified Radial Diffusion for Increased Sensitivity

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In the radial diffusion assay, each molecule of tannin migrates through the gel until it encounters free protein and precipitates. The sensitivity of the method can be increased by decreasing the amount of protein in the gel, since if there is less protein in the gel some molecules of tannin will have to migrate further before encountering free protein and precipitating. Although the ring size is increased by decreasing protein concentrations, the ring intensity is diminished so with this modified method it is usually necessary to stain the gel to make the rings visible.

Method

Use the same protocol as in the normal radial diffusion assay but use only 0.01 g BSA for 100 mL of agar solution. The remainder of the method is exactly as for the normal radial diffusion assay.

Staining

1. After the plates have incubated at 30°C for 96 h, remove them from the incubator. The staining steps include a wash step which removes unprecipitated protein from the gel and a Prussian blue staining step.
2. Mark the gels so that the samples can be identified after removing the gels from the Petri dishes. We mark the gels using a small gauge needle and India ink. Simply dip the needle in the ink, then pierce the gel with the needle. A small black mark will appear in the gel. We mark near sample well #1 with one mark and near sample well #2 with two marks, so the orientation of the gel is unambiguous.
3. Remove the gels from the Petri dishes, using filter paper to support the gel so it doesn't break during handling. Take a piece of dry filter paper that just fits into the dishes (we cut 9 cm paper down to size) and place it over the gel. With your fingers, flatten the paper so it contacts the entire gel. Then loosen the gel from the edge with a spatula, and pry the gel and paper out of the dish. Place the gel into a larger dish for staining, and remove and discard the paper.
4. Wash each gel with 40 mL of 0.3 M NaCl (35.2 g/2L water) for 20 min, shaking continuously. Discard the saline and rinse the gel twice with 10 mL of saline. Repeat the entire wash procedure 4 times. This removes unprecipitated protein from the gel so that the tannin-protein complexes can be visualized.
5. Stain each gel with 20 mL of Prussian blue reagent. Make the reagent fresh daily by mixing equal volumes of 0.10 M $\text{FeNH}_4(\text{SO}_4)_2$ in 0.10 M HCl and 0.008 M $\text{K}_3\text{Fe}(\text{CN})_6$. Stain for several minutes; the tannin-containing rings of precipitate will stain dark blue or blue-green. Pour off the excess stain, and rinse the gel with 0.1 N HCl. Measure the rings immediately; the entire gel eventually turns blue.