BIOLOGY ASSAYS & PROTOCOLS

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Congo Red Assay

Congo Red birefringence assay was developed for the examination of *in vivo* amyloid and was later applied in the examination of *in vitro* samples. Many things other than amyloid exhibit birefringence; buffer salts (phosphate and urea) are inherently birefringent and appear as intense white birefringence, hair or other fibers exhibit birefringence but are often multicolored. This technique is relatively subjective and a known fibrillar sample should be used as a control.

Congo Red spectrophotometric assay is more objective, less prone to misinterpretation, and can be easily combined with the microscopic analysis.

Procedures for Congo Red birefringence assay:

1. Prepare the staining solution: prepare a solution of 80% EtOH: 20% DDI water and add a saturating amount of NaCl. Stir this solution for a few minutes and filter away the excess NaCl. Add a saturating amount of Congo Red, stir, and filter to obtain thefinal working solution. Congo Red can be used straight from the manufacturer or can be recrystallized from 50% EtOH: 50% DDI water prior to use. The staining solution should be used immediately or stored in 0.05% sodiumazide to inhibit bacterial growth.

2. Air-dry 10μL of the aggregated protein solution onto a glass microscope slide.

3. Place 200–400μL of the staining solution onto dried protein sample. Wait for seconds and then blot away the excess solution with a lint-free Kimwipe(or hardened filter paper), being careful not to touch the sample, and allow the stained sample to dry at room temperature.

4. Examine the stained sample using polarized light microscopy.The microscope should have high quality,strain-free lenses for optimal performance. If the polarizersare aligned, the material stained with Congo Red will appear reddish pink (the affinity for CongoRed is known as congophilia). If the polarizers are crossed at a 90┐ angle to each other, the background of the sample will turn black. Any bright spots that appear are a result of birefringence (the sample bends the light in such a way that it can pass through the upper polarizer to reach your eye).The detection of yellow/green birefringence is considered a positive result for the presence of amyloid.The absence of such birefringence is a negativeresult. The birefringence under crossed polarizers should match the areas of Congo Red staining observed under visible light.

Procedures for Congo Red spectroscopic assay:

1. Prepare a 7 mg/mL solution of Congo Red in buffer (5mM potassium phosphate, 150mM NaCl, pH7.4) and filter through a 0.2μm syringe filter immediately prior to use.

2. Zero a UV–Vis spectrophotometer between 400 and700 nm at room temperature with a sample of 1mL phosphate buffer in a disposable cuvette.

3. Add 5μL of the Congo Red solution to the phosphatebuffer, scan between 400 and 700 nm and recordthe spectrum.

4. Add 5–10μL of protein solution to the cuvette and incubate for 30 min at room temperature. At this stage, a red precipitate may become visible. Mix the contents of the cuvette by pipetting the solution upand down and then record the spectrum between400 and 700 nm.

5. Mathematically subtract the Congo Red spectrum from the protein+ Congo Red spectrum. A maximal spectral difference at 540nm is indicative of amyloid fibrils.

6. To use this sample for the microscopic analysis, transfer the protein + Congo Red solution from the cuvette to a centrifuge tube, centrifuge at 12,000–14,000 rpm to pellet the fibrils, wash the fibrils with water, resuspend the fibrils in a small amount of water, and place on a glass microscope slide. Air-dry the sample and analyze under polarized light.

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