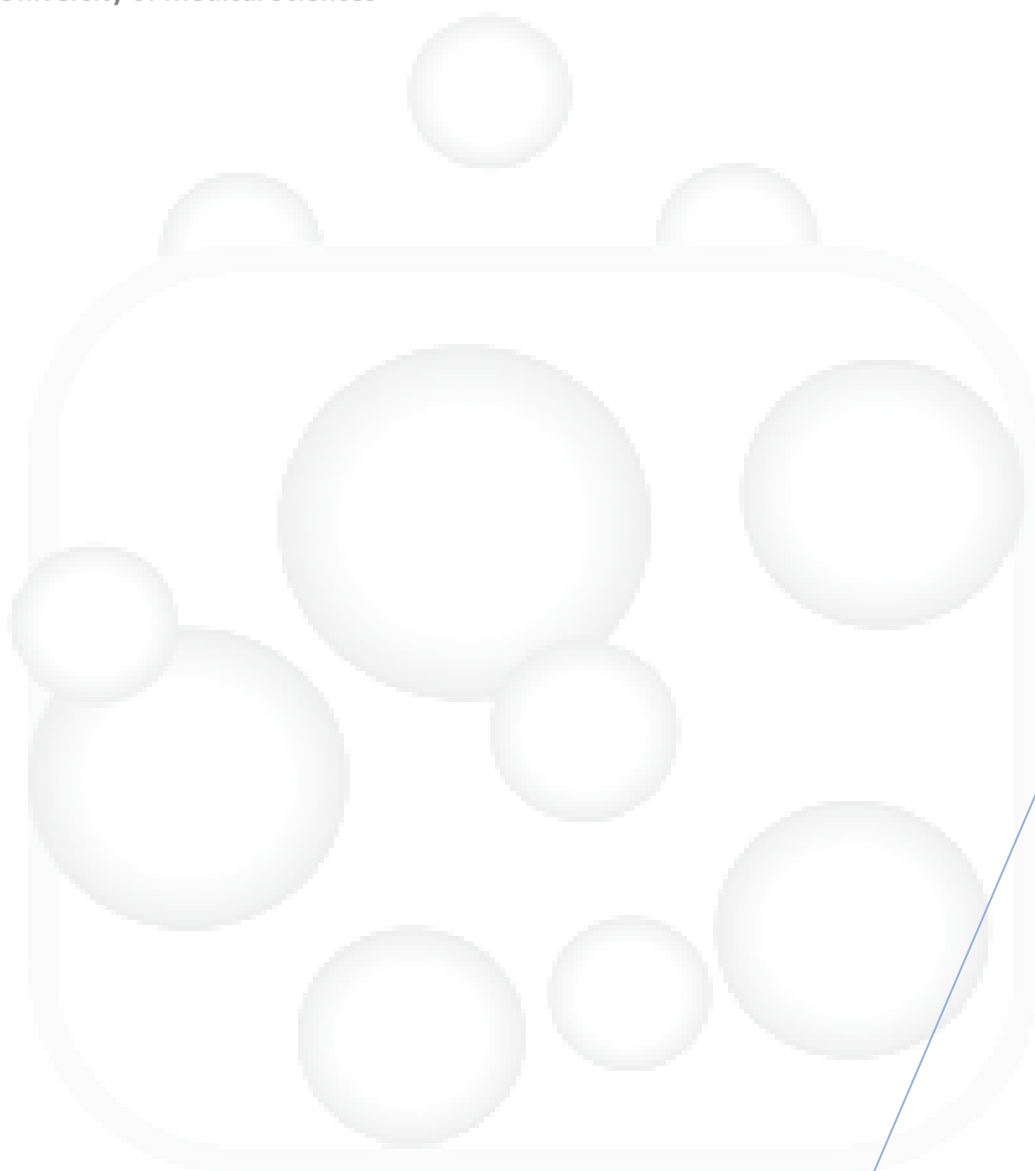




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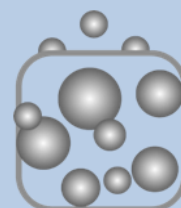
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DNA Ladder Assay

PSR

DNA ladder assay is a method for observing apoptotic DNA fragmentation and DNA fragments are visualized under gel documentation system.

Dr. Rahbar saadat. Y
6/12/2016



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Detailed procedure

1. Aspirate off the media and add 800 μ L Lysis buffer.
2. Transfer the lysis buffer/cell lysate into a 2 mL Eppendrof tube.
3. Incubate tubes at 65°C for 5 min.
4. Add an equal volume of chloroform/isoamyl alcohol to each tube and mix gently.
5. Centrifuge at 12,000 rpm for 5 min.
6. At this point, there will be three layers (phases) in each tube: carefully transfer the aqueous (upper) phase to a new 1.5 ml Eppendorf.
7. Add 700 μ L of cold isopropanol and mix gently, place in -70°C for 30 min to 2 hrs.
8. Centrifuge at 12,000 rpm for 5 min, pour off the tubes and let the pellets air-dry.
9. Add 50 μ L deionized distilled water to each tube. Then measure the absorbance. The 260/280 ratio should be greater than 1.8.
10. Load the DNA samples on a 1.5% agarose gel containing 1 μ L/100mL SYBR Safe DNA gel stain.
11. Examine the gel by an ultraviolet gel documentation system.

Reference

Rahbar Saadat Y., Saeidi N., Zununi Vahed S., Barzegari A., Barar J. An update to DNA ladder assay for apoptosis detection. BioImpacts, 2015, 5(1), 25-28.

Good luck!



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