Alternative protocol for DNA fragmentation analysis

Procedure

1. Pellet cells.
2. Lyse cells in 0.5ml Detergent Buffer: 10 mm Tris (pH 7.4), 5 mm EDTA, 0.2% Triton.
3. Vortex.
4. Incubate on Ice for 30 min.
5. Centrifuge at 27000 g for 30 min.
6. Divide supernatants into 2-250 ul aliquots.
7. Add 50 ul ice cold 5 M NaCl to each followed by vortexing.
8. Precipitate DNA: Add 0.6 ml 100% EtOH and 150 ul 3 M NaAcetate, pH 7.2 place at –80°C freezer for 1 hr. Centrifuge 20,000 g for 20 min and pool DNA extracts by re-dissolving by adding 400 ul total) of extraction buffer (10 mM Tris and 5 mm EDTA).
9. Add 2 ul (10 mg/ml) DNase free RNase. Incubate for 5 hr at 37°C.
10. Add 25 ul Proteinase K at 20 mg/ml and 40 ul of buffer (100 mm Tris, pH 8.0, 100 mM EDTA, 250 mM NaCl. Incubate overnight at 65°C.
11. Extract DNA with phenol, chloroform, isoamyl alcohol and precipitate with EtOH.

From Kotamraju et al JBC, 2000. DNA Fragmentation:

A distinctive feature of apoptosis at the biochemical level is DNA fragmentation. This method was used as a semi-quantitative method for measuring apoptosis. The culture medium was removed and centrifuged at 3000 × g for 5 min to collect detached cells. Adherent cells were lysed with a hypotonic lysis buffer (10 mM Tris-HCl, pH 8.0) containing EDTA (10 mM) and Triton X-100 (0.5%) and then pooled with pellets made of detached cells. RNA was digested using RNase (0.1 mg/ml at 37 °C for 1 h) followed by proteinase K treatment for 2 h at 50 °C. DNA was extracted with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1). DNA was precipitated by adding an equal volume of isopropyl alcohol, stored overnight at 20 °C, and centrifuged at 12,000 × g for 15 min at 4 °C. The pellet was air-dried, resuspended in 20 µl Tris acetate EDTA buffer supplemented with 2 µl of sample buffer (0.25% bromphenol blue, 30% glyceric acid), and electrophoretically separated on a 2% agarose gel containing 1 µg/ml ethidium bromide and visualized under ultraviolet transillumination.