MEASUREMENT OF RADIOACTIVITY IN NUCLEIC ACIDS

Precipitation with Trichloroacetic Acid (TCA)

1. Spot a known volume (up to 10 μl) of the sample to be assayed onto the center of a Whatman GF/C glass-fiber disc (2.4 cm diameter).

2. Add an equal volume of the sample to a tube containing 100 μl of a solution of salmon sperm DNA (500 μg/ml in 20 mM EDTA). Add 5 ml of ice-cold 10% TCA, mix, and chill on ice for 15 minutes.

3. Collect the precipitate by filtering the solution through another GF/C glass-fiber disc. Wash the filter six times with 5 ml of ice-cold 10% TCA, followed by 5 ml of 95% ethanol.

4. Dry both filters under a heat lamp. Put filters into scintillation vials. Count in a liquid scintillation counter in a toluene-based scintillation fluid such as Omnifluor (New England Nuclear). The first filter measures the total radioactivity in the sample; the second filter measures the radioactivity incorporated into nucleic acids. Nucleic acids greater than 20 nucleotides in length are quantitatively precipitated by this procedure.

Note

TCA is prepared as described on page 447.

Absorption to DE-81 Filters

1. Spot a known volume (up to 5 μl) onto the center of each of two 2.4-cm discs of Whatman DE-81 paper.

2. Wash one of the discs six times, 5 minutes per wash in 0.5 M Na_2HPO_4. Then wash the disc twice in water (1 minute per wash) and twice in 95% ethanol (1 minute per wash).

3. Dry both filters under a heat lamp. Count in a liquid scintillation counter in an aqueous scintillation fluid such as Aquasol (New England Nuclear). The unwashed filter measures the total radioactivity in the sample. The washed filter measures only the radioactivity incorporated into nucleic acids.