Sterilizing Nutrient Media

Two methods (autoclaving and membrane filtration under positive pressure) are commonly used to sterilize culture media. Culture media, distilled water, and other stable mixtures can be autoclaved in glass containers that are sealed with cotton plugs, aluminum foil, or plastic closures. However, solutions that contain heat-labile components must be filter-sterilized.

Generally, nutrient or plant tissue culture media are autoclaved at 1.05 kg/cm^2 (15 psi) and 121 °C. This high temperature not only kills bacteria and fungi, but also their heat-resistant spores. Media can be sterilized in either an autoclave or pressure cooker with similar results. The time required for sterilization depends upon the volume of medium in the vessel. For small volumes of liquids (100 ml or less), the time required for autoclaving is 15-20 min, but for larger quantities (2-4 liter), 30-40 min is required. The pressure should not exceed 20 psi, as higher pressures may lead to the decomposition of carbohydrates and other thermolabile components of a medium. There is evidence that medium exposed to temperatures in excess of 121 °C may not properly gel or may result in poor cell growth. The minimum times required for sterilization of different volumes of medium are listed below.

Since many proteins, vitamins, amino acids, plant extracts, hormones, and carbohydrates are thermolabile and may decompose during autoclaving, filter sterilization may be required. The porosity of the filter membrane should be no larger than 0.2 microns (μm). Empty glassware that is to hold media must be sterilized in an autoclave before filter sterilization.

Nutrient media that contain thermolabile components can be prepared in several steps. That is, a solution of the heat-stable components is sterilized in the usual way by autoclaving, and then cooled to 35 - 50 °C under sterile conditions; in a separate operation, solutions of the thermolabile components are filter-sterilized. The sterilized solutions are then combined under aseptic conditions to give the complete media.

MINIMUM AUTOCLAVING TIME FOR PLANT TISSUE CULTURE MEDIA

Volume of Medium per Vessel (mL)	Minimum Autoclaving (min)	Volume of Medium per Vessel (mL)	Minimum Autoclaving (min)
25	20	500	35
50	25	1000	40
100	28	2000	48
250	31	4000	63

Please Note: Minimum Autoclaving time includes the time required for the liquid volume to reach the sterilizing temperature (121°C) and 15 min at 121°C (Burger, 1988). Times may vary due to differences in autoclaves. Validation with your system is recommended.

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