QUANTITATION OF RESIDUAL DNA

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I. INTRODUCTION

This procedure describes a quantitation test for residual DNA. Low levels of DNA can copurify with biological products isolated from living systems. Quantitation of the level of DNA, as an impurity, in a purified product such as proteins and polysaccharides is important for monitoring process consistency.

Hoechst 33258 dye (bis benzimidazole) in solution has an excitation wavelength of 356 nm and an emission wavelength of 492 nm. When the dye binds to DNA the excitation is 365 nm while the emission is 458 nm. The DyNA Quant™ 200 Fluorometer is optimized to detect the bound form of the dye. The A-T (Adenine-Thymine) content of the DNA determines the amount of fluorescence. Calf Thymus DNA is used as the standard and contains about 60% A-T. RNA does not compete for the dye with DNA since the A-T pair is not found in RNA. Single stranded DNA has a fluorescence of about half that of double-stranded DNA. This method assumes what is measured is doubled-stranded.

II. MATERIALS

A. Equipment (those listed or equivalent)

1. **Fluorometer** (Hoefer DyNA Quant™ 200) (**Do Not Substitute**)
2. **Standard assay cuvette** (Hoefer TKO 105) or equivalent
3. **pH Meter** (Orion Model 410A)
4. **Beckman DU640I Series Spectrophotometer**
5. **Analytical balance** (Sartorius)