荧光法测定 DNA 浓度

Measurement of DNA concentration using PanVera fluorescence assay

Steve Hahn

Last updated 9/30/98

This Dye measures DNA concentration using a fluorescent dye which binds only double stranded DNA. The fluorescence of this dye is greatly amplified when bound to DNA.

1. Turn on the Beacon 2000 instrument and allow it to warm up for 30 min to 1 hr before taking any measurements.

2. To generate a standard curve, aliquot the following amounts of DNA to eppendorf tubes:

30 ng, 20 ng, 10 ng, 5 ng, 2.5 ng, 1 ng, 0.5 ng. Also needed are 2 tubes with no DNA. One tube will receive dye and the other will receive no dye (the blank).

The DNA can be diluted in TE, but the maximum volume added to the assay tubes should be 5 microliters or less.

3. Aliquot amounts of the unknown DNA to tubes in the range of the standard curve (preferably in the midpoint of the range).

4. Add 200 microliters of PanVera DNA assay buffer to each tube and mix well (Important: the DNA assay buffer must be used, H20 or other buffers will not work).

5. Prepare and label glass tubes for fluorescence measurement in the Beacon 2000.

6. Add 6 microliters PanVera DNA assay dye to each reaction and vortex (the dye can be added to all the tubes and then all samples read; the values do not significantly

change within 10 min of dye addition).

7. Measure the total intensity value within ~10 min of adding the dye. Use program #3 (static measurement with single blank value at 23 degrees).

8. The standard curve should be linear. Determine the unknown DNA concentrations from the standard curve.