

Genomic DNA Quantitation using the Picofluor

Assay Solutions

1. Make 1X TNE using 10X stock (see pg. 2 for stocks) and filtered dH₂O (50 ml usually sufficient)
2. Make 2X and 1X Hoechst 33258 assay solutions
 - a. Dilute 15 μ l Hoechst 33258 working solution (1 mg/ml) into 7.5 ml 1X TNE.
 - i. Final Hoechst = 2 μ g/ml = 2X
 - b. Dilute 15 μ l Hoechst 33258 (1 mg/ml) into 15 ml 1X TNE.
 - i. Final Hoechst = 1 μ g/ml = 1X

Standard Curve Dilutions

3. Dilute standard curve samples (Calf Thymus DNA) into 1X Hoechst)
 - a. Stock Calf Thymus DNA (CTD) at 0.1 mg/ml = 100 μ g/ml
 - i. 10000 ng/ml (50 μ l CTD + 200 μ l TNE + 250 μ l **2X** Hoechst)
 - ii. 5000 ng/ml (250 #i + 250 **1X** Hoechst)
 - iii. 2500 ng/ml (250 #ii + 250 **1X** Hoechst)
 - iv. 1000 ng/ml (50 #i + 450 **1X** Hoechst)
 - v. 500 ng/ml (250 #iv + 250 **1X** Hoechst)
 - vi. 0 ng/ml (1X Hoechst)

Unknowns Dilutions

4. Dilute unknown genomic DNAs in 1X Hoechst into range covered by standard curve
 - a. e.g. 1 μ l into 199 μ l 1X Hoechst for samples in the range of 0.01 – 2 mg/ml
5. Pipette 100-200 μ l each sample into fluorimeter cuvettes. Avoid bubbles!

Picofluor Setup & Calibration

6. Turn on & toggle to UV channel by pressing [A/B]
7. Press [STD VAL] and use up or down arrows to set at 500.
8. Press [CAL] and follow instructions:
 - a. Press [ENTER]
 - b. Insert blank (sample vi) then press [ENTER]
 - c. Insert 500 ng/ml sample (v) as reference then press [ENTER].
 - i. The 500 ng/ml sample is now set for a reading of 100.

Sample Readings & Calculations

9. Take readings for Standard Curve (including samples vi and iv) and genomic DNAs.
 - a. Read samples by inserting cuvette and pressing [READ]. Fluorescence values are expressed relative to the reference sample – e.g. sample v should read around 500, sample ii should read around 5000.
10. Plot standard curve values on a linear plot with ng/ml on Y axis and Fluorescence on X axis. Curve fit to obtain equation and R² value.
 - a. NOTE: Careful dilution should produce a curve with R² >0.99
11. Use standard curve equation to calculate ng/ml value for genomic DNAs. Don't forget to account for dilution factor.
 - a. NOTE: If the highest accuracy is not needed, you can translate fluorescence readings directly into ng/ml under this setup.

Reagents

10X TNE

100 mM Tris-HCl (12.11 g Tris base/L, MW=121.14)
10 mM EDTA (3.72 g/L, MW=372.20)
2M NaCl (116.89 g/L, MW=58.44)
pH 7.4
0.45 or 0.2 µM filter

Hoechst 33258

20 mg/ml stock solution (Chemical Room -20 deg.)
Make 1 mg/ml working solution in dH₂O – can be stored in dark at 4 deg. ~1-3 mo.

Calf Thymus DNA Stock

Sigma product D-4764
Resuspend in 1X TNE at 0.1 mg/ml final concentration
Stock solution in Chemical Room -20 deg.

Mini cuvettes for fluorimeter

Additional Reading

(These PDF files are also in the Fluorimeter folder on the snap server).

http://www.turnerdesigns.com/t2/doc/appnotes/PDF/998_3617.pdf

<http://www.turnerdesigns.com/t2/doc/appnotes/PDF/S-0046.pdf>