DNA genotek

DNA quantification of Oragene®/saliva samples† using SYBR® Green I Dye and a micro-plate reader

Introduction

Measurement of absorbance at 260 nm (A_{260}) is commonly used for quantifying DNA. Disadvantages of using A_{260} include (i) insensitivity of the assay, and (ii) interference by non-DNA components such as RNA, particularly in samples that are not highly purified. This assay, developed by DNA Genotek, uses SYBR Green I, a fluorescent dye which has specificity for double-stranded (ds)DNA. The purpose of this protocol is to quantify purified total dsDNA by fluorescence.

Equipment and reagents

- Micro-plate reader (e.g., TECAN – M200 96-well plate reader)
- 96-well black plate (e.g., Greiner – 96-well plate, black. VWR Cat. No. 82050-784)
- 1x TE (10 mM Tris HCI, 1 mM EDTA, pH 8)
  - Store at room temperature
- SYBR Green I dye (Invitrogen Cat. No. S7563)
  - 100x working stock prepared by diluting 10 µL of SYBR Green I dye with 990 µL TE buffer
  - Store in 10 or 20 µL aliquots in 0.2 mL PCR tubes at -20°C
  - Before each use, thaw at room temperature. Discard unused portion
- dsDNA for Standard Curve – Lambda DNA (Invitrogen Cat. No. 25250-010)
  - Serially dilute the DNA to give a total of 7 dilutions plus a no-DNA point
  - 50 µL aliquots of each standard are stored in 0.2 mL PCR tubes at - 20°C (See Table 2. Standards A-H)
  - Thaw one tube of each standard

Procedure

1. Preparation of master mix

Prepare a master mix solution, sufficient for all tubes to be assayed.

	Volume (µL)	20 + n n = number of unknown samples
TE	94	
100x SYBR Green 1	1	
Total	95	

Table 1

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

2. Standard curve

For each standard (A-H):

a. In duplicate, add 5 µL of each standard to a well of a black 96-well plate.
b. Add 95 µL of the master mix to each well.

Standard	Concentration (ng/µL)	Volume (µL)	Total DNA (ng)
A	10.0	5	50.0
B	5.0	5	25.0
C	2.50	5	12.5
D	1.25	5	6.25
E	0.625	5	3.12
F	0.3125	5	1.56
G	0.156	5	0.78
H	0.0	5	0.0

Table 2

3. Unknown purified saliva samples

For each unknown sample (n):

a. Dilute purified DNA 1:50 in 1x TE (4 µL sample + 196 µL 1x TE).
b. Add 5 µL of unknown sample to a well of a black 96-well plate.
c. Add 95 µL of master mix.

4. Read fluorescence of samples

a. Excitation 485 nM.
b. Emission 535 nM.

Technical support is available Monday to Friday (9h00 to 17h00 EST):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
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