DNA quantitative assay using Fluorometry

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Abstract

The protocol follow the instruction manual from BIO-RAD of Catalog number 170-2480

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Protocol

Step 1.
Preparation of dye solutions:
Dilute the 10 mg/ml hoechst 33258 to 2 ug/ml for following steps, the ratio for dilution can follow the table:

1 mg/ml Hoechst 33258

10 mg/ml hoechst 33258	10 ul
sterile water	90 ul

2 ug/ml Hoechst 33258

1 mg/ml hoechst 33258	6 ul
10x TEN assay buffer	30 ul
sterile water	270 ul

NOTES
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The mixture of 1 mg/ml Hoechst should be store in the dark at 4 degree, and the 2 ug/ml one should be make fresh before use and place in teh dark

Step 2.
Preparation of DNA standard:
1) Diulte the 1 mg/ml calf thymus DNA to 100 ug/ml and 10 ug/ml respectively, the ratio for dilution can follow the table:

100 ug/ml DNA standard

1 mg/ml calf thymus DNA	10 ul
10x TEN assay buffer	10 ul
sterile water	80 ul

10 ug/ml DNA standard

1 mg/ml calf thymus DNA	10 ul
10x TEN assay buffer	100 ul
sterile water	890 ul

NOTES

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The mixture of DNA standard should be stored at 4 degree

Step 3.

Loading the microplate

1) Pipet 200 ul Hoechst 33258 dye to each well;

2) Add different amount calf thymus DNA to wells and mix the solutions using a disposable transfer pipet for each well;

3)Add the sample into wells and mix the solutions well

4) Measure the fluorescence using an excitation filter of 360-390nm and an emission filter of 450-470nm**.**

Cuvette	Total DNA	DNA Stock Solution	DNA Volume	2 µg/ml Hoechst Dye
1	2000 ng	1 mg/ml	2 µl	200 µl
2	1000 ng	100 µg/ml	10 µl	200 µl
3	500 ng	100 µg/ml	5 µl	200 µl
4	100 ng	10 µg/ml	10 µl	200 µl
5	50 ng	10 µg/ml	5 µl	200 µl
6	20 ng	10 µg/ml	2 µl	200 µl
7	Blank	-—	-—	0