DNA QUANTITATION

WITH THE HOEFER TKO 100 FLUOROMETER

By MES

INTRODUCTION

The TKO 100 Mini-fluorometer is designed to quantify low concentrations of DNA. DNA quantitation with the TKO 100 is based on the binding of bis-benzimidazole (Hoechst 33258) to the DNA. The fluorometer is optimized to detect the bound form of the dye and measures in relative fluorescence units, rather than absolute units. The fluorometer is calibrated to a DNA sample of known concentration and once calibrated, concentrations of unknown samples of DNA are displayed in the same units[1] (ug/ml or ng/ul).

For troubleshooting or unit maintenance, see operating manual[1].

Caution: Wear gloves when preparing Concentrated Dye Stock, Assay Solutions and throughout protocol.

I EQUIPMENT

TKO 100 Mini-Fluorometer	Lens tissue
Cuvette	Parafilm
2.0 ul pipettor	Repipet Jr.
0.1-10 ul pipette tips	Gloves
Kimwipes	Distilled water

II SOLUTIONS

A. **10X TNE** Concentrated buffer, pH 7.4

For 250 ml:

Into 200ml distilled water, add:	Final conc:

3.02 g Tris base	100 mM Tris
5 ml 0.5 M EDTA stock	10 mM EDTA
29.2 g NaCl	2.0 M NaCl

Adjust pH to 7.4 with HCl.

Add distilled water to 250ml.

Autoclave. Store 4^{o}C up to 12 months.

B. **1X TNE** Working Buffer, pH 7.4

(10 mM Tris, 1 mM EDTA, 0.2M NaCl, pH 7.4)

Dilute Concentrate 1:10 in distilled water. Prepare 200 ml for use in Repipet Jr.

C. **Concentrated Dye Stock** (1 mg/ml in H_2O)

Hoechst 33258 binds DNA and **caution** should be used when making this solution. Supervison is strongly recommended when learning to prepare this solution.

Hoechst 33258	10 mg
Distilled, filtered H_2O	10 ml

Store 4^{o}C, protected from light, up to 6 months.

D. Assay Solutions:

Keep at room temperature and protected from light.

i. **Assay solution A** (Standard Assay for DNA samples 10 ng/ml to 500 ng/ml)

(0.1 ug/ml Dye Stock in Working Buffer)

200 ml 1x TNE Working Buffer

20 ul 1 mg/ml Concentrated Dye Stock

Assay solution B (Extended Range Assay for DNA samples 100 ng/ml to 2000 ng/ml)

(1.0 ug/ml Dye Stock in Working Buffer)

200 ml 1x TNE Working Buffer

200 ul 1 mg/ml Concentrated Dye Stock

E. **Sheared Salmon Sperm DNA Reference Standard** (100 ug/ml)

Vortex thawed concentrate (10 mg/ml) very well before use.

890 ul sterile distilled H_2O

100 ul 10x TNE

10 ul Sheared Salmon Sperm DNA concentrate

Store 100-ul aliquots at -20^{o}C.

III PROTOCOL

Turn on fluorometer at least 15 min. prior to use.

Adjust SCALE control on fluorometer to 50% sensitivity (5 clockwise turns from fully counterclockwise position).

A. Standard Assay

i. Thaw Sheared Salmon Sperm DNA Reference Standard and vortex well (for approximately one minute) prior to use.

ii. Dispense 2 ml Assay Solution A (Repipet Jr.) into cuvette. Wipe cuvette with lens tissue (NOT kimwipe), and then insert cuvette into fluorometer well orienting etched marking facing you. Close the well lid.

iii. Zero the fluorometer by turning the ZERO control until the display reads “000.” The reading may flicker +/- 3 units while

on 50% sensitivity.

iv. Remove the cuvette from the well and pipette 2 ul of the DNA Reference Standard into the Assay Solution. Cover the cuvette with Parafilm and mix well by inverting the cuvette several times. Return the cuvette to the well and close the lid.

    1. 2 ul of 100ug/ ml DNA Reference Standard in 2 ml Assay Solution A results in a concentration of 100 ng/ml.

v. Set the scale for the reference standard:

    1. For genomic DNA, turn the SCALE knob until the display reads 100, indicating 100 ng/ml final concentration in the cuvette or 100 ug/ml (100 ng/ ul) undiluted DNA Reference Standard.

    2. For plasmid and fosmid circular DNA, set the SCALE to read “126.”

vi. Repeat steps ii-v to assure that the reference standard readings are reproducible.

vii. Measure unknown samples in the same manner as steps ii-v above. Readings for the concentrated samples are read in ng/ul or ug/ml.

    1. For accuracy, zeroing the instrument is recommended each time you load 2 ml Assay Solution. When processing a great number of samples, zeroing may be performed every five to ten samples . DO NOT adjust the SCALE knob.

    2. Always close the lid between sample measurements.

    3. Rinse cuvette with distilled water and blot dry on Kimwipes between samples.

B. Extended Range Assay

Proceed as for Standard Assay with the following modifications:

i. Use a 1 mg/ml DNA reference standard.

2 ul of 1 mg/ ml DNA Reference Standard in 2 ml Assay Solution B results in a concentration of 1000 ng/ml.

ii. Use 2 ml Assay Solution B for zeroing the fluorometer, setting the scale and for measuring unknown samples.

iii. Set the scale for the reference standard:

1. Turn the SCALE knob until the display reads 1000; indicating 1000 ng/ml final concentration in the cuvette or 1000 ug/ml (1000 ng/ ul) undiluted DNA Reference Standard.

1. Hoefer Scientific Instruments, 1993. "Operating Instructions TKO 100 Mini-Fluorometer," 20788/Rev C/3-05-93.