

# DNA Quantification Using the Fluorescence/DNase (F/D) Assay

## Introduction

The F/D assay is more accurate than absorbance at 260 nm for quantifying the amount of double-stranded (ds) DNA in an unknown sample. There are two steps in the F/D assay. First, an aliquot of DNA is mixed with a fluorescent dye that preferentially binds dsDNA. The amount of fluorescence is proportional to the amount of DNA.

The second step uses pancreatic deoxyribonuclease enzyme (DNase I) to digest all of the DNA in a separate aliquot. Since all of the DNA has been digested, the remaining fluorescence is a measure of the non-DNA background. The total amount of DNA can then be determined by subtracting the background fluorescence from the total fluorescence and comparing this value to a standard curve prepared from pure genomic DNA.

## Equipment and stock reagents to be supplied by user

Equipment and stock reagents	Specifications
Pancreatic deoxyribonuclease (DNase I) (200 ×)	<ul style="list-style-type: none"> <li>Dissolve 5 mg per mL of 1× DNase I buffer</li> <li>Store frozen</li> </ul>
DNase I buffer (20 ×)	<ul style="list-style-type: none"> <li>100 mM MgCl<sub>2</sub>; 20 mM CaCl<sub>2</sub>; 400 mM Tris-HCl, pH 7.5</li> <li>Store frozen</li> </ul>
SYBR Green I dye	<ul style="list-style-type: none"> <li>Supplier: Molecular Probes, Inc.</li> <li>Stock is 10,000 ×</li> <li>Before each use, dilute 1:100 in water to 100 ×</li> </ul>
Stock dsDNA Standard (125 µg/mL)	<ul style="list-style-type: none"> <li>Purified DNA prepared from white blood cells or salmon sperm is preferred because both contain very little RNA</li> <li>A commercial DNA Standard may also be used</li> <li>Shearing DNA by brief sonication will make it easier to dissolve when frozen stock is thawed</li> <li>DNA Standard at a concentration of 125 µg/mL will have an A<sub>260</sub> of 2.500, too high to read directly. A 1:5 dilution should have an A<sub>260</sub> of 0.500.</li> <li>Store at -20°C in 50 µL aliquots</li> </ul>
EDTA (0.5 M)	
Fluorimeter or fluorescence microtiter plate reader	<ul style="list-style-type: none"> <li>Excitation wavelength = 497 nm</li> <li>Emission wavelength = 520 nm</li> </ul>

## DNA Quantification Protocol

### Notes:

- This protocol assumes the use of 1mL samples read in a fluorimeter. All volumes may be reduced proportionately if using a fluorescence microtiter plate reader.
- We recommend running all samples in duplicate for better accuracy.



**(A) Prepare a Working Solution of the Stock dsDNA Standard**

1. Thaw a 50  $\mu\text{L}$  aliquot of the Stock dsDNA Standard (125  $\mu\text{g}/\text{mL}$ ) at 37°C for at least 30 minutes to allow time for the DNA to dissolve completely.
2. Add 450  $\mu\text{L}$  water (1:10 dilution) to the aliquot.
3. The final concentration of the Working dsDNA Solution is now 12.5  $\mu\text{g}/\text{mL}$  (12.5  $\text{ng}/\mu\text{L}$ ).

**(B) Prepare a standard curve**

1. Using the Working dsDNA Solution, prepare the dsDNA standards as described in the chart below.

Tube #	Water ( $\mu\text{L}$ )	20 $\times$ DNase I buffer ( $\mu\text{L}$ )	Working dsDNA Solution ( $\mu\text{L}$ )	Final DNA concentration ( $\text{ng}/\text{mL}$ )
S1	925	50	0	0
S2	923	50	2	25
S3	921	50	4	50
S4	917	50	8	100
S5	909	50	16	200
S6	893	50	32	400
S7	877	50	48	600

2. Add water, 20  $\times$  DNase I buffer, and Working dsDNA solution in the order shown. Mix well.

**(C) Prepare unknown DNA samples**

1. For each unknown DNA sample, prepare Tubes #T1 (no DNase) and D1 (with DNase) as described in the chart below.

Tube #	Water ( $\mu\text{L}$ )	20 $\times$ DNase I buffer ( $\mu\text{L}$ )	Unknown DNA sample ( $\mu\text{L}$ )	200 $\times$ DNase I ( $\mu\text{L}$ )	Dilution factor
T1	921	50	4	0	1:250
D1	916	50	4	5	1:250

2. Add water, 20  $\times$  DNase I buffer, unknown DNA sample, and 200  $\times$  DNase I in each tube in the order shown. Mix well.

**(D) Incubation and Reading of Fluorescence**

1. Incubate all of the tubes (standards and unknowns) at 37°C for 40 minutes.
2. Add 20  $\mu\text{L}$  of 0.5 M EDTA to all tubes (to stop the DNase I digestion reaction).
3. Add 5  $\mu\text{L}$  of 100  $\times$  SYBR Green I dye to each of the tubes. Mix well.
4. Transfer samples to a fluorimeter cuvette or to a microtiter plate.
5. Set excitation to 497 nm and emission detection to 520 nm.
6. Read fluorescence values within 30 minutes of adding the dye.

**Calculate the amount of DNA in the unknown DNA samples**

1. DNA-specific fluorescence = (T1. Total fluorescence) – (D1. Fluorescence after DNase I digestion)
2. Determine the quantity of DNA by extrapolating from the standard curve.
3. Remember to account for the dilution factor (1:250) when calculating the total amount of DNA in the original sample.