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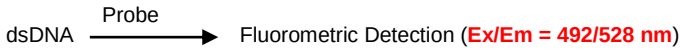
DNA Quantification Assay Kit (Fluorometric)

12/18

(Catalog #K539-200, -2000; 200/2000 assays; Store at -20°C)

I. Introduction:

DNA quantification is essential for a variety of applications, such as real-time PCR (rtPCR), qPCR, Next Generation Sequencing (NGS), etc. The measurement of DNA using analytical instruments (i.e. "nanodrop") is often limited by the presence/contamination of other macromolecules like protein and RNAs found in biological samples. However, the development of highly specific probes that bind to DNA allows the measurement of this macromolecule using a fluorimeter. The biggest advantages of this approach is protein, RNA and single-stranded DNA (ssDNA) are not detected. BioVision's DNA Quantification Assay Kit provides a quick, specific, and easy method for the measurement of DNA concentrations in a wide variety of samples. In this assay, DNA specifically reacts with a probe producing a stable fluorescence signal (Ex/Em = 492/528 nm). The kit is simple to perform, specific, sensitive and high-throughput adaptable with a wide detection range (0.4-150 ng DNA) and a limit of detection of 4 pg/µl dsDNA in samples.



II. Applications:

- Quantification of DNA for rtPCR, qPCR, Next Generation Sequencing (NGS)

III. Sample Type:

- Purified DNA samples

IV. Kit Contents:

Components	K539-200	K539-2000	Cap Code	Part Number
10X DNA Buffer	20 ml	50 ml	WM/NM	K539-XXX(X)-1
200X DNA Probe (in DMSO)	100 µl	1 ml	Red	K539-XXX(X)-2
λDNA Standard (3 ng/µl)	1 ml	10 ml	Blue/NM	K539-XXX(X)-3

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Molecular Biology Grade water

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **10X DNA Buffer:** Warm to room temperature before use. Store at 4 ºC or -20 ºC.
- **200X DNA Probe (in DMSO):** Warm to room temperature before use. Keep away from light. Store at -20 ºC
- **λDNA Standard:** Warm to room temperature before use. Store at -20 ºC

VII. DNA Quantification Protocol:

1. **DNA Samples:** Prepare 1X DNA buffer. (Add 500 µl of 10X DNA buffer into 4.5 ml dH_2O.) Prepare/isolate DNA sample using preferred protocol from biological source (cell, tissue, blood, mitochondria, and bacteria, etc.). Add 2-100 µl of the DNA samples into each of the sample wells. Make up the volume to 100 µl with 1X DNA buffer.

   Note: For unknown samples, prepare different wells with different concentrations to ensure that the concentrations fall within the range of the standard curve.

2. **Preparation of DNA Standards: For wide range DNA concentration application:** Prepare DNA Standards as suggested in the table below by diluting λDNA standard using 1X DNA buffer in plates. For a wide range DNA concentrations (4 -150 ng/well DNA), prepare standards as below:

Vial	Volume of λDNA Standard (µl)	Volume of 1X DNA buffer (µl)	Final λDNA concentration (ng/well)
1	110 of 3 ng/µl stock	110	150
2	110 from Vial 1	110	75
3	110 from Vial 2	110	37.5
4	110 from Vial 3	110	18.75
5	110 from Vial 4	110	9.38
6	110 from Vial 5	110	4.69
7	110 from Vial 6	110	2.35
8	0	110	0

Add 100 µl of each vial into separate wells of a 96-well black plate.

For low DNA concentrations: Make 200 pg/µl λDNA standards by adding 3 µl of the 3 ng/µl λDNA stock into 42 µl of dH_2O. Add 0, 2, 4, 6, 8, 10 µl of the 200 pg/µl λDNA working solutions into a series of wells, generating 0, 400, 800, 1200, 1600, 2000 pg of DNA/well. Adjust the volume to 100 µl/well with 1X DNA buffer.

3. **DNA Probe:** Prepare enough DNA probe by diluting the 200X DNA Probe stock (e.g. add 10 µl of 200X DNA Probe stock into 1990 µl of 1X DNA buffer). Add 100 µl of the 1X DNA Probe into each well containing DNA standards and DNA samples. Mix well, cover the plate and incubate for 5 min at **25 °C** and protected from light.

   Note: Working solutions should be prepared prior to running your experiments, protected from light and *should be used within 4 hours.*

4. **Measurement:** Measure fluorescence (Ex/Em = 492/528nm) in a microplate reader in endpoint mode.

5. **Calculation:** Subtract 0 standard readings from all standard readings. Plot the wide range and low DNA concentration standard curves separately. Apply fluorescence from DNA samples to the DNA standard curves to get **B** ng of DNA in the sample well.

Sample DNA Concentration = $\frac{B}{V} \times D$ (ng/ µl)

Where: V is the volume of sample added to the well (in µl)

D is the sample dilution factor (if applicable, D=1 for undiluted samples)

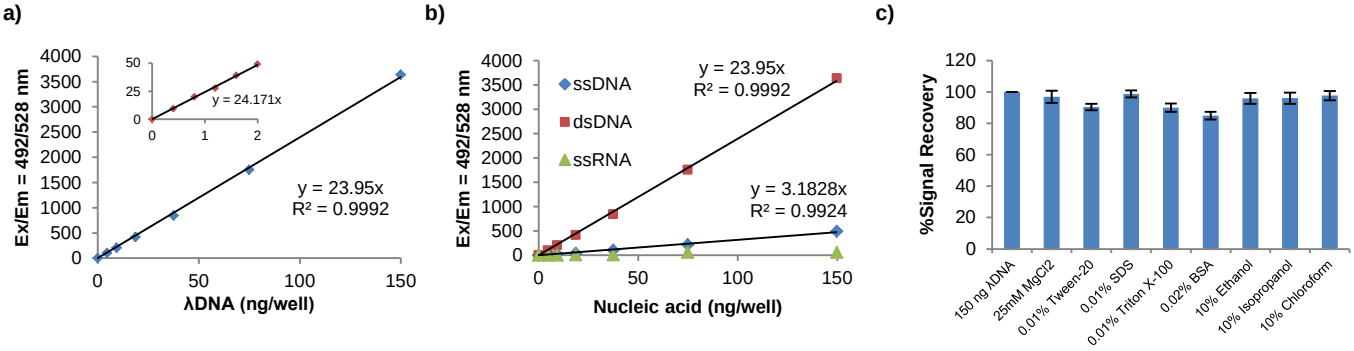


Figure: (a) λDNA standard curve; (b) Specificity of the assay: the probe specifically recognizes dsDNA only. ssDNA and RNA are not detected. (c) 150 ng/well of λDNA mixed with different concentrations of inorganic and organic contaminants commonly found in DNA samples ([contaminants] based on a 10 µl of DNA samples/well). Assays were performed following the kit protocols.

VIII. RELATED PRODUCTS:

Bacterial Genomic DNA Isolation Kit (K309)
DNA Damage Quantification Colorimetric Kit (K253)
DNA Cleanup Maxi Kit (K1369)
Genomic DNA Isolation Kit (K281)
Mitochondrial DNA Isolation Kit (K280)

FOR RESEARCH USE ONLY! Not to be used on humans.