Version 1 updated 2 April 2019

ab252898 DNA Quantification Assay Kit (Fluorometric)

View DNA Quantification Assay Kit (Fluorometric) datasheet:

http://www.abcam.com/ab252898

[use http://www.abcam.cn/ab252898 for China, or http://www.abcam.co.jp/ab252898 for Japan)

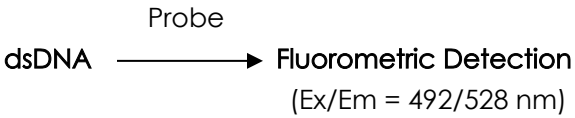
For the quantification of DNA for rtPCR, qPCR and Next Generation Sequencing (NGS).

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

DNA Quantification Assay Kit (Fluorometric) (ab252898) 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.



2. Protocol Summary

Prepare DNA Samples and DNA Standards as directed.

Prepare Probe working solution
(protect from light and use within 4 hrs).

Add Probe working solution (100 µL) to DNA Samples (100 µL) and DNA standards (100 µL).

Incubate for 5 mins at 25°C and protected from light.

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

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components.

Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X DNA Buffer	50 mL	-20°C	-20°C or 4°C
200X DNA Probe (in DMSO)	1 mL	-20°C	-20°C
Lambda DNA Standard	10 mL	-20°C	-20°C

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescent microplate reader capable of measuring Ex/Em = 492/528 nm.
- 96-well plate black microplate with flat bottom.
- Molecular biology grade water.

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 10X DNA Buffer

Warm to room temperature before use. Store at 4°C or -20°C. Dilute 10-fold with dH_2O (e.g. 500 µL 10X DNA buffer plus 4.5 mL dH_2O).

6.2 200X DNA Probe

Warm to room temperature before use. Keep away from light. Store at -20°C.

6.3 Lambda DNA Standard

Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

For normal DNA concentration:

7.1 Prepare DNA Standards as suggested in the table below by diluting Lambda DNA standard using 1X DNA buffer in plates.

7.2 For a wide range of DNA concentrations (4 -150 ng/well DNA), prepare standards as below:

Standard #	Lambda DNA Standard (µL)	1X DNA Buffer (µL)	End amount Lambda DNA in well (ng/well)
1	110 of stock	110	150
2	110 of #1	110	75
3	110 of #2	110	37.5
4	110 of #3	110	18.75
5	110 of #4	110	9.38
6	110 of #5	110	4.69
7	110 of #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:

7.3 Make 200 pg/µL Lambda DNA standards by adding 3 µL of the 3 ng/µL DNA stock into 42 µL of dH2O.

7.4 Add 0, 2, 4, 6, 8, 10 µL of the 200 pg/µL Lambda DNA 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.

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

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8.1 DNA samples:

8.1.1 Prepare/isolate DNA sample using preferred protocol from biological source (cell, tissue, blood, mitochondria, and bacteria, etc.).

8.1.2 Add 2-100 µL of the DNA samples into each of the sample wells.

8.1.3 Make up the volume to 100 µL with 1X DNA buffer.

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

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

9.1 Incubation with DNA probe:

1. 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.
2. Add 100 µL of the 1X DNA Probe into each well containing DNA standards and DNA samples.
3. Mix well, cover the plate and incubate for 5 minutes at 25°C and protected from light.

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

9.2 Measurement:

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

9.3 Calculation:

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

Sample DNA concentration = (B/V) x D (ng/µL)

Where:
B = DNA in the sample well from the Standard Curve (ng).
V = sample volume added into the reaction well (µL).
D = sample dilution factor (D = 1, if undiluted).

10. Typical Data

Data provided **for demonstration purposes only.**

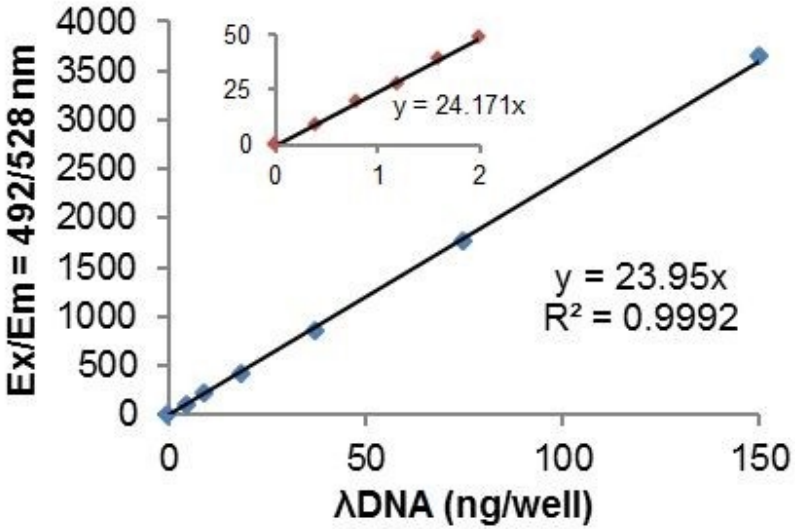

Figure 1. Lambda DNA standard curve.

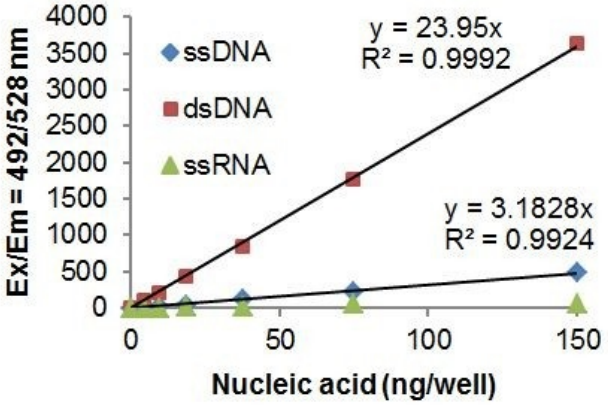

Figure 2. Assay specificity. The probe specifically recognizes dsDNA only. ssDNA and RNA are not detected.

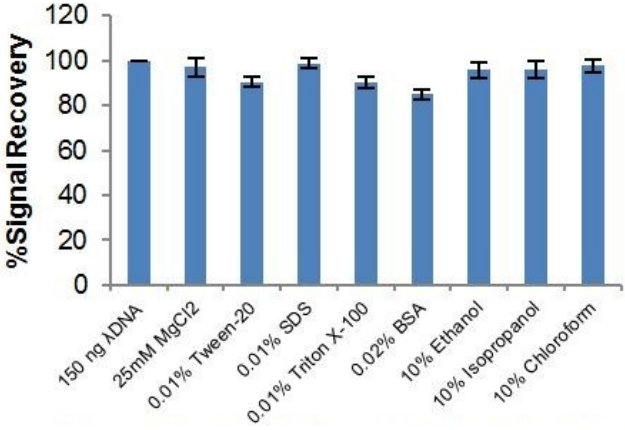

Figure 3. Effect of contaminants. Lambda DNA (150 ng/well) mixed with different concentrations of inorganic and organic contaminants commonly found in DNA samples; ([contaminants] based on 10 µL of DNA sample/well).

11. Notes

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