ab156898

Circulating DNA Quantification Kit

Instructions for Use

For the quantification of circulating DNA from plasma and serum.

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

Version 5 Last Updated 31 July 2018

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1. Background

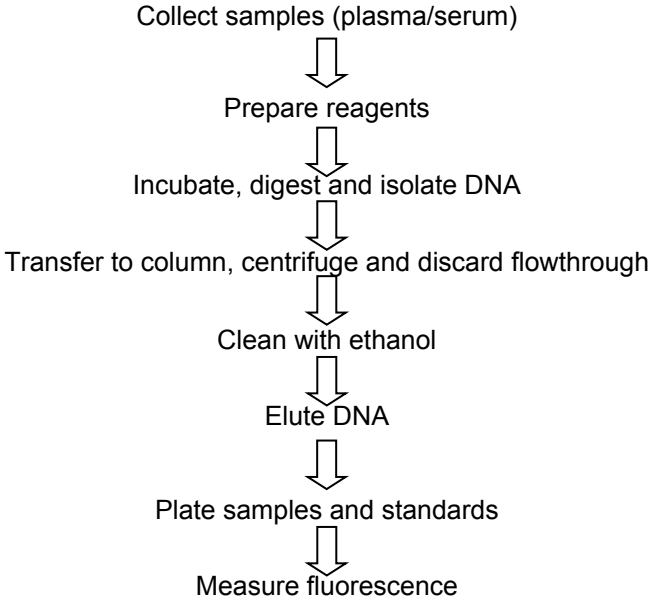
Increased amounts of circulating DNA have been found in a variety of disorders including cancer, autoimmune diseases, and infections. The measurement of circulating DNA has important implications for the diagnosis, prognostication, and monitoring of these disorders. The measurement of circulating DNA has also found potential application in the post-treatment monitoring of transplant patients and the assessment and prognostication of trauma patients. Accurate quantification of circulating DNA concentration, especially when DNA is present at low concentrations, is critical for detecting and discriminating the disorders. Meanwhile, a rapid and convenient assay method would enable the determination of circulating DNA to be easily performed.

Abcam's Circulating DNA Quantification Kit (ab156898) provides a rapid and convenient method for measuring circulating DNA. The kit has the following features:

- Very fast procedure. The assay can be finished within 30 minutes.
- Simple and convenient. Reagents for convenient DNA isolation and purification are ready in the kit. No extra DNA isolation and purification system is required.
- Sensitive and accurate. Linear detection range 0.1 ng to 100 ng (1-1000 ng/mL) in 96-well plate assay.
- No interference. Fluorescence is only from purified DNA.

ab156898 is suitable for quantifying circulating DNA from plasma and serum by applying our proprietary DNA isolation buffer to samples. After treatment with DNA digestion buffer, the DNA is recovered with our specially designed Fast-Spin Column. DNA is then fluorescently quantified.

2. Assay Summary



3. Materials Supplied

Item	48 tests	96 tests	Storage (Before Preparation)
DNQ1 (DNA Digestion Solution)	1 mL	2 mL	RT
DNQ2 (DNA Digestion Powder)	1 vial	2 vials	–20°C
DNQ3 (DNA Isolation Buffer)	15 mL	30 mL	RT
DNQ4 (DNA Elution Solution)	1 mL	2 mL	RT
DNQ5 (50X DNA Assay Solution)	0.25 mL	0.5 mL	–20°C
DNQ6 (Assay Dilution Buffer)	12 mL	24 mL	RT
DNQ7 (DNA Standard, 10 µg/mL)*	0.1 mL	0.2 mL	4°C
F-Spin Column	50	100	RT
F-Collection Tube	50	100	RT

4. Storage and Stability

Upon receipt: (1) Store DNQ2 at –20°C, or store it at 4°C as soon as it is dissolved in DNQ1; (2) Store DNQ5 at –20°C away from light, (3) Store DNQ7 at 4°C; (4) Store all other components at room temperature (15-25°C).

**For maximum recovery of the products, centrifuge the original vial prior to opening the cap*.

5. Materials Required, Not Supplied

- Ethanol (70% and 90%)
- TE buffer (pH 7.5)
- Adjustable pipette or multiple-channel pipette
- Aerosol resistant pipette tips
- Microplate reader capable of reading fluorescence Ex/Em = 480/520.

6. Reagent Preparation

a) Prepare 70% Ethanol and 90% Ethanol Solutions:
Add distilled water to the concentrated Ethanol (96-100%) to obtain a 70%and a 90% Ethanol Solution.

b) Prepare DNQ1/DNQ2 DNA Digestion Solution:
Add 1 mL of DNQ1 to DNQ2. Vortex until the solution is clear. The DNQ1/DNQ2 Solution can be stored at 4°C.

c) Prepare 1X DNQ5 DNA Assay Solution:
Dilute DNQ5 (50X DNA Assay Solution) with DNQ6 (Assay Dilution Buffer) to make a 1X DNA Assay Solution (ex. Add 2 µL of DNQ5 to 98 µL of DNQ6).
Note: *Thaw DNQ5 at room temperature for 5-10 minutes prior to use.*

7. Standard Preparations

Suggested Standard Curve Preparation: First, dilute **DNQ7** (DNA Standard) with **DNQ6** at a 1:10 ratio (ex: add 10 µL of **DNQ7** (DNA

Standard) to 90 µL of **DNQ6**). Then, further prepare seven concentrations of DNA Standard by combining **DNQ5, DNQ6 and DNQ7** according to the following dilution chart:

DNQ5 (50X DNA Assay Solution)	**DNQ6** (Dilution Buffer)	**Diluted DNQ7** (DNA Standard)
2 µL	0 µL	100 µL
2 µL	60 µL	40 µL
2 µL	80 µL	20 µL
2 µL	90 µL	10 µL
2 µL	95 µL	5 µL
2 µL	98 µL	2 µL
2 µL	99 µL	1 µL

Add each solution to the wells of 96-well plate for measurement of fluorescence. The final concentration of DNA in the mixed solutions should be 100, 40, 20, 10, 5, 2 and 1 ng/100 µL, respectively (from the top to the bottom).

8. Sample Preparation

Reagents for convenient DNA isolation and purification are ready in the kit. No extra DNA isolation and purification system is required.

9. Assay Procedure

Note: Always close spin columns before placing them in the microcentrifuge.

a) Add 300 µL of DNQ3 (DNA Isolation Buffer) and then 20 µL of the mixed DNQ1/DNQ2 Solution (see Reagent Preparation) to 300 µL of plasma/serum. Mix well and incubate at 65°C for 8-

10 minutes. Meanwhile, place a spin column into a 2 mL collection tube.

b) Transfer mixture to the column. Spin for 30 seconds at 12,000 rpm. Discard the flowthrough. Replace the column to the collection tube (Note: maximum volume of the column is 600 µL).
c) Add 300 µL of 70% ethanol to the column and centrifuge at 12,000 rpm for 20 seconds. Discard the flowthrough and replace the column to the collection tube. Add 200 µL of 90% ethanol to the column and centrifuge at 12,000 rpm for 20 seconds.
d) Discard the flowthrough and replace the column to the collection tube. Add an additional 200 µL of 90% ethanol to the column and centrifuge at 12,000 rpm for 40 seconds.
e) Place the column in a new 1.5 mL vial. Add 8-20 µL of DNQ4 (DNA Elution Solution) directly to the column filter, and centrifuge at 12,000 rpm for 20 seconds to elute DNA.
f) Add 100 µL of the 1X DNA Assay Dilution Buffer (see Reagent Preparation) to each well of a 96-well plate followed by adding 2-10 µL of purified DNA sample. Mix lightly. For negative control, add 2-10 µl of 1 X TE (pH7.5) instead of sample. For standard curve preparation, see above.
g) Incubate for for 5-10 min at room temperature, protected from light, and measure fluorescence (∆F) at Ex = 480-500 nm and Em = 520-550 nm using a fluorescence microplate reader. Signal is stable for about 2 hours.

10. Data Analysis

Calculation of DNA concentration: Plot ∆F value versus amount of standard DNA and determine the slope as ∆F/ng.
Calculate DNA concentration of sample using the following formula:

$$DNA\ conc\ (ng/mL) = \frac{sample\Delta F - blank\Delta F}{slope} \times sample\ dilution$$

Typical standard curve

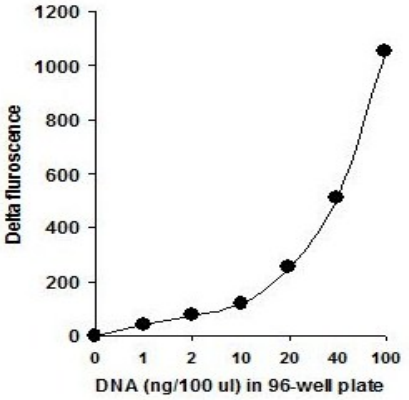


Figure 1. Typical standard curve generated using the Circulating DNA Quantification Kit (ab156898).

UK, EU and ROW
Email: technical@abcam.com | Tel: +44(0)1223696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp