FitAmp™ Circulating DNA Quantification Kit

Base Catalog # P-1012

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *FitAmp*™ Circulating DNA Quantification Kit is suitable for quantifying circulating DNA from plasma and serum.

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KIT CONTENTS

Components	48 assays P-1012-1	96 assays P-1012-2
CD1 (DNA Digestion Solution)	1 ml	2 ml
CD2 (DNA Digestion Powder)	1 vial	2 vials
CD3 (DNA Isolation Buffer)	15 ml	30 ml
CD4 (DNA Elution Solution)	1 ml	2 ml
CD5 (50X DNA Assay Solution)**	0.25 ml	0.5 ml
CD6 (Assay Dilution Buffer)	12 ml	24 ml
CD7 (DNA Standard, 10 μg/ml)*	0.1 ml	0.2 ml
F-Spin Column	50	100
F-Collection Tube	50	100

** For maximum recovery of the products, centrifuge the original vial prior to opening the cap.*
*** Thaw at room temperature for 5-10 minutes prior to use.*

SHIPPING & STORAGE

Upon receipt: (1) store **CD2** at –20°C, or store it at 4°C as soon as it's dissolved in **CD1**; (2) store **CD5** at –20°C away from light; (3) store **CD7** at 4°C; (4) store all other components at room temperature (15-25°C). When stored properly, the kit components are stable for up to 6 months.

GENERAL PRODUCT INFORMATION

Quality Control: EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The *FitAmp*™ Circulating DNA Quantification Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: *FitAmp*™ is a trademark of EpigenTek, Inc.

A BRIEF OVERVIEW

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

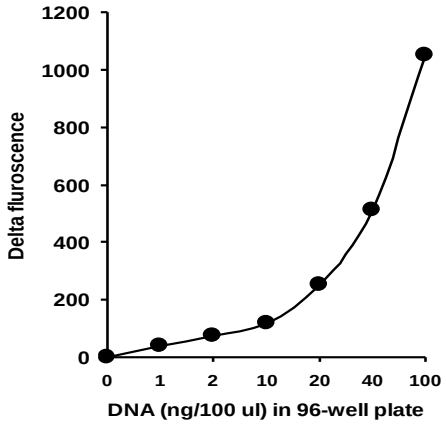
convenient assay method would enable the determination of circulating DNA to be easily performed.

EpigenTek's *FitAmp™* Circulating DNA Quantification Kit 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 & accurate. Linear detection range 0.1 ng - 100 ng (1-1000 ng/ml) in 96-well plate.
- No interference. Fluorescence is only from purified DNA.

PRINCIPLE & PROCEDURE

The FitAmp™ Circulating DNA Quantification Kit simply applies our proprietary DNA isolation buffer to plasma/serum. After treatment with DNA digestion buffer, the DNA is recovered with our specially designed Fast-Spin Column. DNA is then fluorescently quantified.



Human placenta DNA was added into microplate wells at different concentrations and quantified using the FitAmp™ Circulating DNA Quantification Kit.

PROTOCOL

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

Before starting, prepare the required solutions (not included): 90% ethanol and 70% ethanol.

1. Add 1 ml of CD1 to CD2. Vortex until solution is clear. Add 300 µl of CD3 and then 20 µl of the mixed CD1/CD2 Solution to 300 µl plasma/serum. Mix well and incubate at 65°C for 8-10 minutes. Meanwhile, place a spin column into a 2 ml collection tube.
2. 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.)*
3. 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.

4. 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.
5. Place the column in a new 1.5 ml vial. Add 8-20 µl of **CD4** directly to the column filter, and centrifuge at 12,000 rpm for 20 seconds to elute DNA.
6. Dilute **CD5** 50X DNA Assay Solution with **CD6** Assay Dilution Buffer to make a 1X concentration of **DNA Assay Solution** (ex: add 2 µl of **CD5** to 98 µl of **CD6**).
7. Add 100 µl of the new **1X DNA Assay Solution** 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, see *Preparation of Standard Curve* on next page.
8. Incubate for 5-10 min at room temperature, protected from light, and measure fluorescence (ΔF) at Ex 480-500 and Em 520-550 nm using a fluorescence microplate reader. Signal is stable for about 2 hours.

Calculation: Plot ΔF value versus amount of standard DNA and determine the slope as ΔF/ng.

Calculate DNA concentration of sample using the following formula:

$$\text{DNA concentration (ng/ml)} = \frac{\text{sample } \Delta F - \text{blank } \Delta F}{\text{Sample volume } (\mu l)^* \times \text{slope}} \times 1000$$

PREPARATION OF STANDARD CURVE

Dilute standard DNA with **CD6** at a 1:10 ratio (ex: add 10 µl of DNA standard to 90 µl of **CD6**). Prepare standard curve:

CD6 (µl)	Diluted Standard DNA (µl)	50X DNA Assay Solution (µl)
0	100	2
60	40	2
80	20	2
90	10	2
95	5	2
98	2	2
99	1	2

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).

RELATED PRODUCTS

P-1004 FitAmp™ Plasma/Serum DNA Isolation Kit