FitAmp™ Blood and Cultured Cell DNA Extraction Kit

Base Catalog # P-1018

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *FitAmp*™ Blood and Cultured Cell DNA Extraction Kit is suitable for isolating DNA from blood leukocytes and cultured mammalian cells. Typical yield of DNA isolated from cells using this kit varies depending on the input sample.

The *FitAmp*™ Blood and Cultured Cell DNA Extraction Kit allows isolation of DNA in quantities from 1 ng to 4 μg, optimal at between 10 ng and 1 μg

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KIT CONTENTS

Components	50 samples P-1018-050	100 samples P-1018-100
CB1 (Suspending Buffer)	16 ml	2 x 16 ml
CB2 (DNA Digestion Solution)	1.1 ml	2.2 ml
CB3 (DNA Digestion Powder)	1 vial	2 vials
CB4 (DNA Capture Buffer)	16 ml	2 x 16 ml
CB5 (DNA Elution Solution)	1 ml	2 ml
F-Spin Column	50	100
F-Collection Tube	50	100

SHIPPING & STORAGE

The kit can be stored at room temperature (15-22°C) for up to 6 months from the date of shipment, with the exception of CB3. Upon receipt, CB3 should be stored at –20°C, or stored at 4°C as soon as it is dissolved in CB2 (stable for up to 6 months).

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Waterbath or heat block
- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Pipettes and pipette tips
- ☐ 15 ml conical tube
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Ethanol (96-100%)
- ☐ Lymphoprep solution

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*™ kits are for research use only and are not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

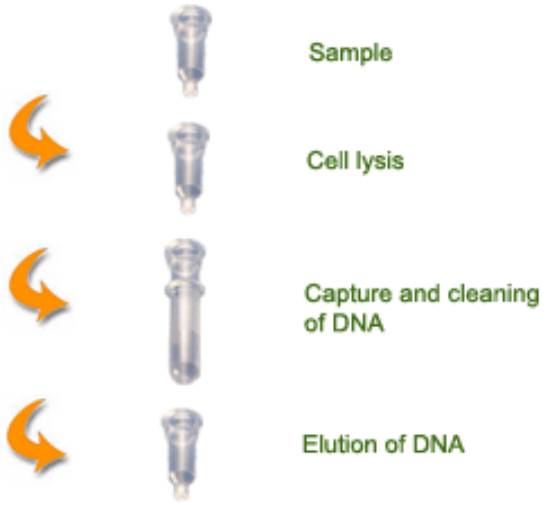
The *FitAmp*™ Blood and Cultured Cell DNA Extraction Kit is designed for rapid isolation of pure genomic DNA from blood leukocytes or cultured mammalian cells in a small amount. The extracted DNA can be used for any molecular biology procedures such as PCR, restriction digestion, cloning and sequencing, etc. DNA yield can be up to 4 μg from 10^6 blood leukocytes or cultured mammalian cells.

The *FitAmp*™ Blood and Cultured Cell DNA Extraction Kit has the following features:

- The fastest procedure available, which can be finished within 20 minutes with consistent isolation conditions.
- High efficiency of DNA isolation from blood leukocytes or cultured mammalian cells.
- Use of non-toxic reagents and no phenol chloroform.

PRINCIPLE & PROCEDURE

The *FitAmp*™ Blood and Cultured Cell DNA Extraction Kit simply applies our proprietary DNA isolation buffer to cell pellets. After treatment with the DNA digestion buffer, DNA is easily recovered in quantities of 10-20 μl by our specially designed F-Spin Column. DNA is ready for downstream applications.



Schematic Procedure for Using the *FitAmp*™ Blood and Cultured Cell DNA Extraction Kit

PROTOCOL

Note: When processing spin columns, always cap spin columns before placing them in the microcentrifuge.

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

1. Cell collection: **For blood**, leukocytes can be separated with a standard leukocyte isolation method, or according to the following procedure: 3 ml of EDTA-treated peripheral blood are laid over 1.5 ml of lymphoprep 1.077 ($^{w}/_{v}$) in a 15 ml tube. The tube is then centrifuged at 3000 rpm for 15 minutes. An individual band (containing about 1×10^6 leukocytes) is transferred into a 1.5 ml vial from the gradient. Centrifuge the cells at 2000 rpm for 3 minutes and discard the supernatant. Wash cells with 1 ml of PBS once by centrifugation at 2000 rpm for 3 minutes. **For adhesive cultures**, cells (no more than 1×10^6) are detached by trypsinization and collected into a 1.5 ml vial. Centrifuge the cells at 2000 rpm for 3 minutes and discard the supernatant. Wash cells with 1 ml of PBS once by centrifugation at 2000 rpm for 3 minutes. **For suspension cells**, cells (no more than 1×10^6) are directly collected into a 1.5 ml vial. Centrifuge the cells at 2000 rpm for 3 minutes and discard the supernatant. Wash cells with 1 ml of PBS once by centrifugation at 2000 rpm for 3 minutes.
2. Remove supernatant and add 200 µl of **CB1** to suspend the cell pellet.
3. Add 1 ml of **CB2** to one vial of **CB3**. Vortex until solution is clear. Add 4 µl of the mixed **CB2/CB3 solution** to 200 µl of cell suspension. Vortex and incubate at 65°C for 15 minutes. Meanwhile, place a spin column into a 2 ml collection tube.
4. Add 300 µl of **CB4** to the cell suspension, mix, and transfer to the column. Spin for 45 seconds at 12,000 rpm. Discard the flowthrough. Replace the column to the collection tube. (**Note:** *maximum volume of the column is 600 µl.)*
5. Add 300 µl of *70% ethanol* to the column and centrifuge at 12,000 rpm for 30 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 30 seconds.
6. 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.
7. Place the column in a new 1.5 ml vial. Add 8-18 µl of **CB5** directly to the column filter, and centrifuge at 12,000 rpm for 20 seconds to elute DNA.

DNA is now ready for use or storage at –20°C.

RELATED PRODUCTS

P-1003	*FitAmp*™ Tissue Section DNA Isolation Kit
P-1004	*FitAmp*™ Plasma/Serum DNA Isolation Kit
P-1009	*FitAmp*™ Paraffin Tissue Section DNA Isolation Kit
P-1017	*FitAmp*™ Urine DNA Isolation Kit