ab156900 Genomic DNA Extraction Kit

Instructions for Use

For rapid isolation of small amounts of pure genomic DNA from blood leukocytes or cultured mammalian cells.

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

Version 1 Last Updated 27 February 2013

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1. Background

The Genomic 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.

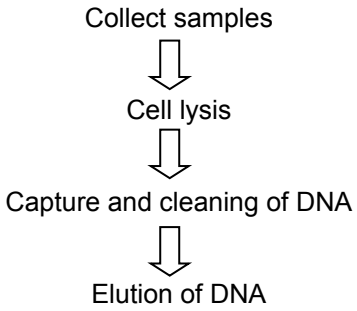
ab156900 has the following features:

- 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.

The typical yield of DNA isolated from cells using this kit varies depending on the input sample. The Genomic DNA Extraction Kit allows isolation of DNA in quantities from 1 ng to 4 µg, optimal at between 10 ng and 1 µg.

The Genomic 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 then ready for downstream applications.

2. Assay Summary



3. Materials Supplied

Item	50 tests	100 tests	Storage (Before Preparation)
DNG1 (Suspending Buffer)	16 mL	2 x 16 mL	RT
DNG2 (DNA Digestion Solution)	1.1 mL	2.2 mL	RT
DNG3 (DNA Digestion Powder)	1 vial	2 vials	–20°C
DNG4 (DNA Capture Buffer)	16 mL	2 x 16 mL	RT
DNG5 (DNA Elution Solution)	1 mL	2 mL	RT
F-Spin Column	50	100	RT
F-Collection Tube	50	100	RT

4. Storage and Stability

ab156900 can be stored at room temperature (15-22°C) with the exception of component DNG3 (DNA Digestion Powder). Upon receipt, DNG3 should be stored at –20°C, or stored at 4°C as soon as it is dissolved in DNG2 (DNA Digestion Solution).

5. Materials Required, 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

6. Reagent Preparation

a) Prepare 90% Ethanol and 70% Ethanol Solutions:
Add distilled water to the concentrated Ethanol (96-100%) (not supplied with ab156899) to obtain a 90% Ethanol Solution and a 70% Ethanol Solution.

b) Prepare DNG2/DNG3 Solution:
Add 1 mL of DNG2 to DNG3. Vortex until the solution is clear. The DNG2/DNG3 Solution can be stored at 4°C for up to 6 months.

7. Sample Preparation

Blood Samples: Leukocytes can be separated with a standard leukocyte isolation method, or according to the following procedure: laid 3 mL of EDTA-treated peripheral blood over 1.5 mL of lymphoprep 1.077 (w/v) in a 15 mL tube. Centrifuge the tube at 3000 rpm for 15 minutes.

An individual band (containing about 1 × 106 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.

Adhesive Cell Cultures: Cells (use ≤ 1 × 106 cells) 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.

Suspension Cell Cultures: Cells (use ≤ 1 × 106 cells) 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.

8. Assay Procedure

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

a) Remove supernatant from sample (prepared as described in Section 7) and add 200 µL of DNG1 (Suspending Buffer) to suspend the cell pellet.
b) Add 4 µL of the mixed DNG2/DNG3 Solution (see Section 6) 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.

c) Add 300 µL of DNG4 (DNA Capture Buffer) 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.*

d) 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.

e) 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.

f) Place the column in a new 1.5 mL vial. Add 8-18 µL of DNG5 (DNA Elution Buffer) 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.

9. Data Analysis

Typical Results

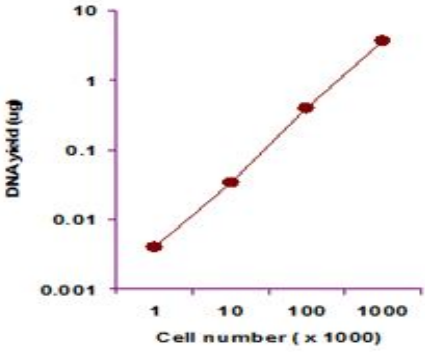


Figure 1. Genomic DNA was isolated from MCF7 cell line using ab156900. The isolated DNA yield was quantified by real time PCR.

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

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