

**ab65358**

# **Genomic DNA Isolation Kit**

## Instructions for Use

For the rapid isolation of Genomic DNA in various cell and tissue samples

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



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# 1. Overview

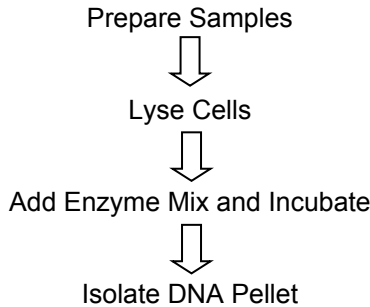
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Abcam's Genomic DNA Isolation Kit provides a simple and convenient procedure for rapid isolation of genomic DNA from mammalian cells and tissue samples with high yield and purity. The novel method requires less than 90 minutes to prepare highly pure genomic DNA.

The extracted genomic DNA is free from protein and RNA, and suitable for a variety of applications such as PCR, DNA hybridization, enzyme manipulation, cloning, Southern blot, and array-based experiments.

# 2. Protocol Summary

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### 3. Components and Storage

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#### A. Kit Components

Item	Quantity
Lysis Buffer III	2 x 1.8 mL
Enzyme B Mix	1 vial
TE Buffer I	1.5 mL

PLEASE NOTE: Lysis Buffer III was previously labelled as Cell Lysis Buffer, and TE Buffer I as TE Buffer, and Enzyme B Mix as Enzyme Mix (Lyophilized). The composition has not changed.

\*Store kit contents at  $-20^{\circ}\text{C}$ . Read the entire protocol before beginning the procedure.

ENZYME B MIX: Add 275  $\mu\text{l}$  of TE Buffer I to Enzyme B Mix, mix well, aliquot and re-freeze immediately at  $-20^{\circ}\text{C}$ . Stable for up to 3 months at  $-20^{\circ}\text{C}$ .

#### B. Additional Materials Required

- Centrifuge and microcentrifuge
- Pipettes and pipette tips
- Absolute ethanol

- Orbital shaker

## 4. Assay Protocol

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**Note:** This protocol is designed for using with  $1-2 \times 10^6$  cells, and generally produces 5-20  $\mu\text{g}$  genomic DNA. If larger amount of DNA is desired, scale up the volumes proportionally.

1. Collect cells ( $1-2 \times 10^6$ ) by centrifugation at  $600 \times g$  for 5 minutes at  $4^\circ\text{C}$ .

**Note:**

For tissue samples, ground freshly excised tissue in liquid nitrogen. Weight  $\sim 5$  mg ground fine tissue powder in a microcentrifuge tube.

2. Add 35  $\mu\text{l}$  of Lysis Buffer III. Mix and keep on ice for 1 minute. Vortex for 5 seconds.
3. Centrifuge in a microcentrifuge tube at top speed for 3 minutes. Remove supernatant. **The pellet is isolated nuclei.**
4. Re-suspend the pellet in 40  $\mu\text{l}$  Lysis Buffer III.
5. Add 5  $\mu\text{l}$  of Enzyme B Mix, pipette several times to mix.
6. Incubate in a  $50^\circ\text{C}$  water bath for 1 hour or until the solution becomes clear.

**Note:**

You may extract the sample using 50  $\mu$ l of Phenol/Chloroform to remove insoluble materials before doing ethanol precipitation (optional).

If isolating DNA for DNA damage quantification, incubate at 37°C for 1-2 hours after adding enzyme mix and extract sample using 50  $\mu$ l of Phenol/Chloroform.

7. Add 100  $\mu$ l absolute ethanol, mix and keep at -20°C for 10 minutes.
8. Centrifuge in a microcentrifuge at top speed for 5 min at room temperature.
9. Remove the supernatant. **The pellet is isolated DNA.**
10. Wash the DNA pellet 2 times with 1 ml of 70% ethanol. Remove the trace amount ethanol using pipette tip. Air dry for 5 min.

**Note:**

Do not completely dry the DNA. It would be difficult to dissolve if it is completely dried.

11. Re-suspend the DNA in 20  $\mu$ l TE Buffer I or water; store the extracted DNA at -20°C for future use.

## **Technical Support**

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