Product Information

Genomic DNA Isolation Kit

I. Kit Contents:

Components	K2118-50 50 assays	Color Color	Part Number
Cell Lysis Buffer	2 x 1.8 ml	Purple	K2118-C-1
Enzyme Mix (lyophilized)	1 vial	Red	K2118-C-2
TE Buffer	1.5 ml	Green	K2118-C-3

Note: Add 150 µl of DMSO, and mix well before use.

II. Introduction:

The Genomic DNA Isolation Kit offers an easy and convenient way for fast extraction of genomic DNA from mammalian cells and tissue samples in high yield and purity and takes less 90 minutes. The isolated genomic DNA excludes from the protein and RNA, and can be utilized in various applications (e.g. PCR, cloning and enzyme manipulation etc.)

III. General Consideration and Reagent Preparation:

Read the entire protocol before beginning the procedure.

After opening the kit, store Enzyme Mix at –70°C. Store buffers at 4°C.

Add 275 µl of TE buffer to Enzyme Mix, mix well, aliquot and refreeze immediately at –70°C. Stable for up to 3 months at –70°C.

Be sure to keep all buffers on ice at all times during the experiment.

The protocol is designed for using with 1 - 2 x 10^6 cells, and generally produces 5 - 20 µg genomic DNA. If larger amount of DNA is desired, scale up the volumes proportionally.

IV. Genomic DNA Isolation Protocol:

1. Collect cells (1 - 2 x 10^6) by centrifugation at 600 x g for 5 min at 4°C.

Note: For tissue samples, ground freshly excised tissue in liquid nitrogen. Weigh ~ 5 mg grounded fine tissue powder in a microcentrifuge tube.

2. Add 35 µl of Cell Lysis Buffer. Mix and keep on ice for 1 min. Vortex for 5 sec.

3. Centrifuge in a microcentrifuge tube at top speed for 3 min. Remove supernatant. The pellet is the isolated nuclei.

4. Resuspend the pellet in 40 µl Cell Lysis Buffer.

5. Add 5 µl of Enzyme Mix, pipet several times to mix.

6. Incubate at 50°C water bath for 1 hr or until the solution becomes clear.

Notes:

a. You may extract the sample using 50 µl of Phenol/Chloroform to remove insoluble materials before doing ethanol precipitation (optional).

b. If isolating DNA for DNA damage quantitation, incubate at 37°C for 1 - 2 hrs after adding enzyme mix and extract sample using 50 µl of Phenol/Chloroform.

7. Add 100 µl 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.

10. Wash the DNA pellet 2 times with 1 ml of 70% ethanol. Remove the trace amount ethanol using pipet 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. Resuspend the DNA in 20 µl TE Buffer or water, store the extracted DNA at -20°C for future use.

Frequently Asked Questions:

1. How can I measure the DNA amount or purity after the last step?

Generally 20 - 50 µg DNA can be obtained following the kit procedure. You can either take 1/3 - 1/2 of the solution and add water to 100 µl to measure it using 100 µl micro-cuvet, or alternatively, dilute the whole amount of DNA into 100 ul or 1 ml cuvet for measuring and then recover it by isopropanol precipitation.

2. Does the kit remove mitochondrial DNA?

The kit does not remove the mitochondrial DNA. If you want to remove mitochondrial DNA, you can lyse the cells in 0.5% NP-40 in Tris Buffer (pH 8.0), then centrifuge and isolate nuclei pellet (mitochondrial DNA will be in supernatant). Then follow the kit procedure.

3. I wanted to clarify whether or not the following product can isloate nuclear DNA from mitochondrial DNA in birds?

We have not tested your specific application. However, the genomic DNA isolation kit can be used in any cells or tissue samples. This kit is different from column based DNA isolation kit. In this kit, proteins or RNAs is degraded and DNA is precipitated. The kit can recover all DNAs from your samples.

4. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

5. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

6. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

7. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20°C. Please refer to the datasheet for storage information and shelf life of each of the components.

8. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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