Thermo SCIENTIFIC

PRODUCT INFORMATION

Genomic DNA Purification Kit

#K0512 **for 100 preps**

Lot: **Expiry Date:**

Store at room temperature

www.thermoscientific.com/fermentas

COMPONENTS

Component	#K0512 100 preps
Lysis Solution	40 ml
Precipitation Solution 10X concentrated solution	8 ml
NaCl Solution 1.2 M sodium chloride	10 ml

WARNINGS AND PRECAUTIONS

When handling blood samples, follow recommended procedures for biohazardous materials.

CERTIFICATE OF ANALYSIS

All components of the kit were functionally tested in the purification of genomic DNA from 0.2 ml of whole blood. The purified DNA was tested in PCR amplification of a 950 bp single-copy gene.

Quality authorized: Jurgita Zilinskiene

DESCRIPTION

The Genomic DNA Purification Kit is a simple and rapid system for high quality genomic DNA purification from various sources including: whole blood, serum, cell lines, bacterial cells, plant and mammalian tissues. The kit is based on selective detergent-mediated DNA precipitation from crude lysate. The entire procedure is rapid – only 20-25 min – with a typical yield of 2-10 µg genomic DNA from 0.2 ml of blood.

High molecular weight genomic DNA purified with the kit is suitable for direct use in all common molecular biology applications: PCR, Thermo Scientific FastDigest and conventional restriction digestion, cloning, DNA sequencing and Southern blot analysis.

SAMPLE PREPARATION

Blood

Whole blood must be collected in EDTA- or citrate-coated tubes to prevent clotting and DNA degradation.
DNA extracted from heparinized blood cannot be used for PCR.
Typically 200 µl of fresh blood is used for DNA isolation with the yield of 2-10 µg. If larger DNA quantity is needed, 500 µl of blood should be lysed with 1 ml of water, leukocytes spun down (5000 rpm (~2500 x g), 2 min) and the pellet resuspended in 200 µl of TE buffer.
If the blood is to be stored for later use it can be left at 2-4°C for no longer than 2 months. For long term storage the samples should be aliquoted in 200µl portions and kept at -20°C.

Sera

Using of fresh sera samples is recommended. If it is impossible, samples should be frozen in aliquots of 200 µl and stored at -20°C.

Cell cultures

Cell culture cells should be collected by centrifugation and resuspended in 200 µl of TE buffer. For the efficient DNA separation from cell components do not use more than $0.4-0.6 \times 10^6$ cells. Usage of thawed samples is not recommended.

Epithelium cells

Epithelium cells should be collected by centrifugation (5000 rpm (~2500 x g), 3 min), supernatant removed, and cell pellet resuspended in 200 µl of TE buffer. Using of thawed samples is not recommended.

Tissues

25-30 mg of mammalian tissue (either fresh or frozen at -70°C until use) or 50-100 mg of plant tissue should be pulverized in liquid nitrogen with mortar and pestle, a powder should be placed in a 1.5 ml microcentrifuge tube and resuspended in 200 µl of TE buffer. Using of thawed samples is not recommended.

Bacterial cultures

Bacterial culture should be centrifuged for 10 min at 5000 x g. 10-20 mg of bacterial cells (either fresh or frozen at -20°C until use) should be placed in a 1.5 ml microcentrifuge tube and resuspended in 200 µl of TE buffer. Using of thawed cells is not recommended.

Note
Multiple freezing / thawing of the samples should be avoided, since each cycle dramatically diminishes the yield of intact DNA.

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PROTOCOL

Step	Procedure
1	Mix 200 µl of sample with 400 µl of lysis solution and incubate at 65°C for 5 min. If a frozen sample is used, lysis solution should be added before the thawing. Then the sample is incubated at 65°C for 10 min with occasional inverting of the tube.
2	Immediately add 600 µl of chloroform, gently emulsify by inversion (3-5 times) and centrifuge the sample at 10,000 rpm for 2 min.
3	Prepare precipitation solution by mixing 720 µl of sterile deionized water with 80 µl of supplied 10X concentrated Precipitation Solution.
4	Transfer the upper aqueous phase containing DNA to a new tube and add 800 µl of freshly prepared precipitation solution, mix gently by several inversions at room temperature for 1-2 min and centrifuge at 10,000 rpm (~9400 x g) for 2 min.
5	Remove supernatant completely (do not dry) and dissolve DNA pellet in 100 µl of NaCl solution by gentle vortexing. **Make sure that the pellet is completely dissolved.**
6	Add 300 µl of cold ethanol, let the DNA precipitate (10 min at -20°C) and spin down (10,000 rpm (~9400 x g), 3-4 min). Remove the ethanol. Wash the pellet once with 70% cold ethanol and dissolve DNA in 100 µl of sterile deionized water by gentle vortexing. ***Note*** • *Due to low amounts of DNA present in serum samples, prolong the DNA precipitation to 1-20 hours at -20°C and reduce the volume of water for DNA dissolving to 20 µl.* • *For PCR applications DNA sample washing with 70% ethanol is not necessary.* • *If RNA-free genomic DNA is necessary, add RNase A, #EN0531 to a final concentration of 0.2 mg/ml after step 5 is completed, mix by vortexing and incubate for 10 min at 37°C.*
7	Measure DNA concentration spectrophotometrically or visually after electrophoresis in agarose gel. Check the absence of RNA and estimate the average length of obtained DNA. Usually 2-10 µg of DNA from 200 µl of fresh blood are obtained with average length of approx. 50 kb. ***Note*** • *In blood samples DNA yield depends on quantity of leukocyte cells.* • *Viral DNA from sera samples is not visible in agarose gel.* • *Use 1-5 µl of DNA solution for 50 µl of PCR.*

TROUBLESHOOTING

Problem	Sample	Possible Cause	Solution
Low yield of DNA	Blood	Sample was frozen and thawed several times. Sample was stored at 2-4°C longer than 2 months. Blood clots were present in the sample.	Take a new sample.
	Cell culture	Too much cells were used for purification, and DNA pellet turned insoluble. Sample was frozen and thawed several times.	Reduce cell quantity by a factor of two or more. Take a new sample.
	Any sample	DNA pellet was not completely dissolved during step 5.	Take a new sample.
Degraded DNA	Any sample	Inappropriate storage conditions of the sample.	*see* SAMPLE PREPARATION section.
RNA contamination present	Tissue, Bacteria, Cell cultures		Add RNase A to a final concentration 0.2 mg/ml to the sample prior to the addition of ethanol (*see* step 6) and incubate for 10 min at 37°C.
	Any sample	Inaccurate handling during stages 3 and 4.	Adjust prepared DNA sample volume to 200 µl with H_2O and repeat all purification steps.
		Not fresh precipitation solution was used.	Use freshly prepared precipitation solution.
Enzymatic reactions not running	Blood	Heparinized blood was used.	Use EDTA or citrate treated blood.
	Any sample	Residual reagents (ethanol, salts, etc.) present in prepared DNA, because of inaccurate handling during step 6.	Precipitate DNA with ethanol, wash with cold 70% ethanol and dissolve in sterile deionized water.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans.

Please refer to www.thermoscientific.com/fermentas for Material Safety Data Sheet of the product.

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