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Blood/Genomic DNA Purification Mini Spin Column Kit

Kit for the purification of genomic DNA from human and animal whole blood and cultured cells

Version: 22042012

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- Chemicals
- Biochemicals
- Cell Culture Products
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- Molecular Biology Products
- PCR
- Proteins and Enzymes
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Related Products

Mini Spin Column DNA Purification Kit	Contents	Cat. No.
Genomic DNA Blood & Cell Cultures Blood and cell culture	**50** reactions **250** reactions	S5375.0050 S5375.0250
Plasmid DNA Kit Purification of plasmid DNA	**50** reactions **250** reactions	S5369.0050 S5369.0250
GEL Extraction Gel extraction of fragments and plasmids	**50** reactions **250** reactions	S5374.0050 S5374.0250
JustSpin GEL Extraction Gel extraction of fragments and plasmids	**50** reactions **250** reactions	S5337.0050 S5337.0250
PCR Kit Purification of PCR products	**50** reactions **250** reactions	S5368.0050 S5368.0250
PSI Clone High Throughput PCR Kit Purification of PCR products	**50** reactions **250** reactions	S5303.0050 S5303.0250

Coming Soon - Related Products

Mini Spin Column DNA Purification Kit	Contents	Cat. No.
Genomic DNA BAC Gram-positive and gram-negative bacteria	**50** reactions **250** reactions	
Genomic DNA Plant Plants and soil	**50** reactions **250** reactions	
Genomic DNA Food Food and feed of plant or animal origin	**50** reactions **250** reactions	
Genomic DNA Tissue Tissue including mouse tail	**50** reactions **250** reactions	

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Storage Conditions and Stability

Spin columns of the kit show full performance for at least 1 year when stored dry at room temperature (18-25°C). Proteinase K is delivered as solution and should be stored at -20°C upon arrival. All other kit components should be stored dry at room temperature. This guarantees performance for 1 year.

Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed. Precipitates in buffers AB and WB do not influence the performance of the kits. Close bottles immediately after use. Take care not to transfer precipitates. Precipitates in buffers PB and PS should be dissolved by warming to 70°C.

Limited License

The purchase price paid for the **Genomic DNA Purification Mini Prep Kit** by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended for internal research only by the purchaser. Furthermore, research only use means that the **Genomic DNA Purification Mini Prep Kit** and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without written approval of the manufacturer.

Separate licenses are available from the manufacturer for the express purpose of non-research use and applications. To inquire about such licenses, or to obtain permission to transfer or use the enclosed material, please contact your local distributor.

Limitations of Product Use

The use of this kit is strictly limited to research purposes. They are not to be applied for any diagnostic, including human, or drug purposes. This also excludes administration to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the regulatory authorities in the country of use. All due care and attention should be exercised in handling of the materials described in this handbook.

Before using a PCR Purification Kit, customers and other users should make their own determination that the product is suitable for intended use. They should ensure that they can use the PCR Purification Kit product safely and legally. This document does not constitute a warranty or assume any liabilities on behalf of the manufacturer except in writing signed by the manufacturer. Unless otherwise agreed in writing, the exclusive remedy for all claims is replacement of the product or refund of the purchase price at manufacturer's option, and in no event shall the manufacturer be liable for special, consequential, incidental, punitive or exemplary damages.

Quality Control

Genaxxon bioscience is dedicated to your success and every batch of this product is tested with an extensive routine procedure to make sure that it meets all your needs. However, it has neither been developed nor tested for a specific application.

We reserve the right to change, alter, or modify our Genomic DNA Purification Mini Prep Kit to enhance its performance and design.

This product is for research use only.

Manual Contents

Introduction

This kit contains all ingredients for the isolation of total nucleic acids from whole blood and cell cultures. The kit contains spin columns, buffers and reagents necessary for cell lysis, DNA binding to the matrix, and washing and elution of DNA into a small volume from the matrix. Each kit contains a manual with detailed protocols of DNA extraction and purification from whole blood and cell culture.

The **Genaxxon bioscience** mini spin column DNA purification kit system is the result of our latest innovative nucleic acid purification technology. The kits represent remarkable features of timesaving, easy, prompt and high yield DNA purification. Basis of the technology is the binding of DNA to negatively charged surfaces. The used matrix has an extremely high DNA binding capacity, and it is this property that is made use of for preparation of high amounts of pure DNA. In contrast to silica-based systems, the Genaxxon bioscience matrix reversibly binds DNA in the presence of multivalent cations at low concentration. Low and high molecular weight cell constituents and salt are removed from the matrix by washing with a specially developed buffer and 70% ethanol. Finally, bound DNA is eluted with a small volume of 10mM Tris/HCl, 1mM EDTA (TE buffer).

DNA is suitable for downstream applications including PCR, hybridization, restriction analysis, cloning and other. Optionally, RNA can be removed by a short incubation with RNase A directly on the column.

The quality of DNA according to spectrophotometry holds the standard for pure DNA solutions. The $A_{260/A280}$ ratio of DNA extracted with the **Genaxxon bioscience whole blood** kit lies between 1.7 and 2.0.

Fresh or frozen blood samples, with or without anti-coagulating agents (e.g. EDTA, heparin, citrate), or up to 10^7 suspension or adherent cells can be used.

Examples of typical DNA yields obtained with this purification procedure are: 2.8µg from whole blood (100µL) and 5.2µg from cell culture (10^6 monkey kidney cells).

Appendix

I) DNA precipitation

- Add sodium acetate (3M, pH7.5) to the DNA-containing solution to a final concentration of 0.3M and mix well. For high recovery of DNA, we recommend the use of glycogen or other commercial precipitation supports.
- Add 2 volumes of chilled ethanol and mix well.
- Recover the precipitated DNA by centrifugation (>16,000xg, 4C, 15 min). Carefully decant the supernatant and wash the pellet with 70% ethanol (fill the tube half way and shortly vortex) by centrifugation (>16,000xg, 4°C, 10 min).
- Carefully decant the supernatant and air-dry the pellet at room temperature.
- Dissolve the DNA in the desired volume of 10mM Tris/HCl, pH8.0 or deionized, sterile water.

II) Agarose gel electrophoresis of prepared DNA

A direct method for testing DNA quality in terms of molecular size and conformation (genomic DNA) is agarose gel electrophoresis. Depending on the amount expected, pipette a 1/10 to 1/50 aliquot of the DNA preparation in a microtube or well of a 96 well plate, fill up with sterile water to 10 or 20µL and add gel loading buffer (e.g. 0.25% bromophenol blue, 30% glycerol). Mix by pipetting and transfer to a gel slot. Run the gel in an electrophoresis buffers (e.g. TAE, 40mM Tris-acetate, 1mM EDTA, pH8.0) at 1-5 V/cm.

III) Photometric determination of DNA concentration and quality

Determination of DNA concentration is done by UV reading at 260 nm and 320 nm (background). Correct measurement is only possible when the DNA is free of RNA and readings are at values between 0.1 and 1 absorption units. DNA preparations should be vortexed shortly and diluted accordingly using 10mM Tris/HCl (pH8.0) or water. As a blank, use buffer EB diluted at the same factor as the DNA sample:

DNA concentration (µg/mL) = (A_{260nm} – A_{320nm}) x 50 (DNA extinction coefficient) x dilution factor

DNA yield (µg) = DNA concentration x sample volume (mL).

A standard procedure of measuring DNA quality is the determination of the absorption quotient (Q) of readings at A_{260nm} and A_{280nm}:

$Q = (A_{260nm} – A_{320nm})/(A_{280nm} – A_{320nm})$

For a pure DNA preparation, Q lies between 1.7 and 2.0.

Troubleshooting

This guide may help solve problems that may arise. Genaxxon bioscience welcomes comments and suggestions for improvement and supplement of our protocols or any hints on other molecular biology applications. The Genaxxon bioscience team is always pleased to answer any of your questions about our products.

Observation	Possible cause	Comments/suggestions
Low or no DNA yield	Buffer AB not added Buffer WB not added	Ensure that buffer AB has been added to and mixed with the lysate. Accordingly, make sure that the column has been washed with buffer WB.
Low DNA recovery and reduced flow through	Membrane clogging	Blood lysate contains too much DNA (highly viscous), especially in case of buffy coat blood. Or blood lysate contains carryover of cell debris. In this case blood should be diluted with PBS, e.g. 1:1. Please try different dilutions.
DNA “smear”	Nuclease activity/ contamination	Upon disintegration of samples, cellular nucleases are released and may degrade genomic DNA. Keep samples chilled until DNA extraction. Whenever possible, fresh samples should be used and processed immediately. Storage at -20°C is possible for several months. Several freeze-thaw cycles should be avoided, because this can result in decreased molecular size of the DNA. Use only sterilized glass and plastic ware in order to avoid nuclease contamination.
Low DNA performance	Salt in eluate	Make sure that you followed all washing steps of the procedure. Eventually repeat 70% ethanol washing.

Contents of the kits

	50 Purifications	250 Purifications
Mini spin columns	50	5 x 50
Collection tubes (1.5mL)	50	5 x 50
Buffer RB	10mL	50mL
Buffer CH	12.5mL	62.5mL
Buffer PB	12.5mL	62.5mL
Buffer AB	12.5mL	62.5mL
Buffer PS	10mL	50mL
Buffer WB	20mL	100mL
Buffer EB	10mL	50mL
Proteinase K (10mg/mL)	1mL	3 x 1.67mL
Manual	1	1

Not included: 70 % ethanol for washing steps and RNase A for removal of RNA.

Safety Information

It is strongly recommended to wear a lab coat, disposable gloves and protective goggles when working with chemicals. More detailed information is available in the material safety data sheets, which can be requested from the manufacturer.

Caution: Do not add bleach or acidic solutions to the waste of sample preparation.

Proteinase K

Proteinase K is a sensitizer. See risk and safety phrases R42/43, S23-24-26-36/37.

RNase A:

Ribonuclease A is a sensitizer. See risk and safety phrases R42/43, S23-24-26-36/37.

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Protocol – whole blood (human and animal)

Important notes: please read before starting

- ! Fresh or frozen blood samples, with or without anti-coagulating agents (e.g. EDTA, heparin, citrate), can be used.
- ! If RNase treatment is desired, mix 10µL of DNase-free RNase A stock solution (10mg/mL, cat. No. S5231) with 200µL buffer RB. Vortex at full speed for a few seconds.
- ! You need a bench top microcentrifuge (12,100xg; > 13,000 rpm), precision pipettes and sterile pipette tips allowing pipetting volumes 1 to 10µL, up to 100µL and up to 1000µL, and sterile 1.5mL or 2mL polypropylene tubes. Prepare an ice bath for blood DNA extraction.
- ! Dissolve precipitates in buffer PS by heating to 70°C.
- ! Heat buffer EB to 70°C.
- ! Have 70% (v/v) ethanol at hand.
- ! SDS precipitates in buffer PB are normal. Dissolve buffer PB by heating to 70°C. Swirl to mix completely. Shortly before use, transfer 200µL buffer PB per each application to a sterile tube (not supplied) and add 20µL Proteinase K, vortex for 5 sec.

  Caution: buffer PB is an irritant.

Approximate time for total nucleic acid preparation: 30 min (human blood)

Procedure

1. Transfer 100-200µL of whole blood to a sterile 1.5mL polypropylene tube (not provided).
2. Add 200µL of buffer PB incl. 20µL Proteinase K. Vortex vigorously for 15 seconds.
3. Incubate at 70 °C for 10 min. Incubation of animal blood (e.g. sheep) should be done at 70°C for 30 min. The solution turns into a dark brownish colour.
4. Add 200µL buffer PS and vortex for 5 sec.
5. Incubate tube at 0°C (ice bath) for 5 min.
6. Centrifuge at 12,100xg (> 13,000 rpm) for 3 min. Transfer the supernatant to a new tube. Add 250µL of buffer AB to the supernatant, vortex for 5 sec. and transfer the extract to a spin column by pipetting.
7. The solution may become turbid as a result of protein precipitation.
8. Centrifuge at 12,100xg (> 13,000 rpm) for 30 sec. Discard flow-through.

At this point nucleic acids bind to the matrix. A series of downstream applications is possible without RNA elimination, including PCR, restriction analysis, and cloning. If you want to quantitate extracted DNA by spectrophotometry or use it for hybridisation, RNA has to be removed. RNA-free DNA preparations are obtained following step 8. If you wish to skip RNase treatment for removal of RNA, proceed with step 9.

transfer up to 10^7 cells

2.000 x g for 5 min

add 250µL **buffer CH**

vortex at max. speed for 5 sec, incubate 5 min

add 200µL **buffer AB**

vortex for 5 sec

transfer the extract to a spin column by pipetting

>13,000 rpm for 30 sec

Optional: 200µL **buffer RB** + 10µL **RNase A**, incubate 5-10 min
add 400µL **buffer WB**

>13,000 rpm for 30 sec

wash 400µL **70% ethanol**

>13,000 rpm for 3 min

add 75-150µL preheated to 70˚C buffer EB in the center, incubate 1 min

>13,000rpm for 1 min

elution of high pure DNA

Procedure

1. Transfer up to 10^7 cells to a sterile polypropylene tube (not provided) and centrifuge at 2,000xg for 5 min.
2. Decant the supernatant, taking care to remove excess liquid from the pellet. This can be done by using a pipette.
3. Add 250µL buffer CH, vortex thoroughly at max. speed for 5 sec and let stand for 5 min.
   Cells grown in 8 to 24 well plates can be lysed directly after removal of medium. For this purpose add 250µL buffer CH and incubate for 5 min under occasional swirling or pipetting in and out several times. Thereafter, pipette lysate in a clean, sterile polypropylene tube. **Caution:** buffer CH is an irritant.
4. Add 200µL buffer AB, vortex for 5 sec and transfer the extract to a spin column by pipetting.
   The solution becomes turbid as a result of protein precipitation.
5. Centrifuge loaded column at 12.100 x g (> 13.000 rpm) for 30 s. Discard flow-through. At this point nucleic acids bind to the matrix. A series of downstream applications is possible without RNA elimination, including PCR, restriction analysis, and cloning. If you want to quantitate extracted DNA by spectrophotometry or use it for hybridization, RNA has to be removed. RNA-free DNA preparations are obtained following step 6. If you wish to skip RNase treatment for removal of RNA, proceed with step 7.
6. Optional: For RNA degradation, pipette 200µL of buffer RB incl. 10µL RNase A into the spin column, close lid and incubate for 5 to 10 min.
7. Add 400µL buffer WB to the spin column. Centrifuge at 12.100 x g (> 13.000 rpm) for 30 s. Discard flow-through.
8. Wash the spin column with 400µL of 70% ethanol by centrifugation at 12,100xg (> 13,000 rpm) for 3 min. Carefully remove tube and discard flow-through.
9. Transfer the column to a supplied 1.5mL tube. Place 75 to 150µL buffer EB preheated to 70°C in the center of the column, close lid and incubate for 1 min. Thereafter, centrifuge at 12,100xg (> 13,000 rpm) for 1 min to elute the DNA.

   Note: The elution volume depends on the sample: If high DNA amounts are expected, a higher elution volume may increase the DNA yield. Generally, 100µL elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated elution with heated buffer EB.

See page 10 for precipitation of DNA (appendix I), analysis of DNA by gel electrophoresis (appendix II) and UV measurement (appendix III).

100-200 µL whole blood
+200µL **buffer PB** + 20µL Proteinase K

vortex 15 sec

10 min at 70˚C or 30 min at 70˚C for animal blood
add 200µL **buffer PS**

vortex for 5 sec

ice bath for 5 min

>13.000 rpm for 3 min

transfer the supernatant to a new tube

add 250µL **buffer AB**

vortex 5 sec

transfer the extract to a spin column by pipetting

>13.000rpm for 30 sec

Optional: 200µL **buffer RB** + 10µL **RNase A**, incubate 5-10min,
add 40µL **buffer WB**

>13.000rpm for 30 sec

wash, 400µL **70% ethanol**

>13.000rpm for 30 sec

add 75-150µL preheated to 70˚C buffer EB in the center, incubate 1 min

>13.000rpm for 1 min

elution of high pure DNA

9. Optional: For RNA degradation, pipette 200 µl of buffer RB incl. 10µL RNase A into the spin column, close lid and incubate for 5 to 10 min.
10. Add 40µL buffer WB to the spin column and centrifuge at 12.100 x g (> 13.000 rpm) for 30 sec. Discard flow-through.
11. Wash the spin column with 400µL of 70% ethanol by centrifugation at 12.100 x g (> 13.000 rpm) for 3 min. Carefully remove tube and discard flow-through.
12. Transfer the column to a supplied 1.5mL tube. Place 75 to 150µL buffer EB
13. preheated to 70°C in the center of the column, close lid and incubate for 1 min. Thereafter, centrifuge at 12.100 x g (> 13.000 rpm) for 1 min to elute the DNA.
14. **Incubate** for 1 min. Finally, centrifuge at ≥13,000 rpm for 1 min to elute genome DNA.

    Note: The elution volume depends on the sample: If high DNA amounts are expected, a higher elution volume may increase the DNA yield. Generally, 100µL elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated elution with heated buffer EB.

See page 10 for precipitation of DNA (appendix I), analysis of DNA by gel electrophoresis (appendix II) and UV measurement (appendix III).

Protocol - Cell culture

Important notes: please read before starting

- ! Cells grown in suspension can be proceeded starting with step 1 below. Cells grown in monolayer can be detached by trypsinisation. For this purpose, remove medium from dish or flask and wash cells with PBS. Remove PBS and add trypsin solution. As soon as cells have detached proceed with step 1 below. Cells scraped off the dish or flask can be proceeded directly as described below. If grown in well plates, remove medium by pipetting and lyse cells directly in wells by starting with step 3 below.
- ! If RNase treatment is desired, mix 10µL of DNase-free RNase A stock solution (10mg/mL) with 200µL buffer RB. Vortex at full speed for a few seconds.
- ! You need a bench top microcentrifuge (12.100 g; > 13.000 rpm), precision pipettes and sterile pipette tips allowing pipetting volumes 1 to 10µL, up to 100µL and up to 1000µL, and sterile 1.5mL or 2mL polypropylene tubes.
- ! Heat buffer EB to 70 C.
- ! Have 70% (v/v) ethanol at hand.

Approximate time for total nucleic acid preparation: 25 min