Geno-Sen's® Genomic DNA Extraction Mini Kit.

Geno-Sen's®

Genomic DNA Extraction Mini Kit

For purification of Genomic DNA from

Serum
plasma
body fluids like C.S.F, urine
Lymphocytes/Buffy Coat
Sputum
Cultured Cells.

Procedure Manual

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Genome Diagnostics Pvt. Ltd.

(An ISO 13485:2003, 9001:2000 Certified Company)

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Contents

Geno-Sen's® Genomic DNA Extraction Mini Kit.

Kit Contents: (Geno Sen's® Genomic DNA Extraction Mini Kit)

S.No	Contents	REF 98021 50 preps	REF 98022 250 preps
1.	Spin Columns	**50**	**250**
2.	Collection Tubes (2 ml) Including the one's fitted in the columns	100+50 fitted with columns	500+250 fitted with columns
3.	Binding Buffer MBD*	12 ml	60 ml
4.	Wash Buffer MWB W1* (concentrate)	20 ml	2 x 50 ml
5.	Wash Buffer MWB W2*† (concentrate)	12.5 ml	2 x 25 ml
6.	Elution Buffer MEL†	10 ml	50 ml
7.	Proteinase K dilution Buffer PKB	1.250 ml	5 x 1.250 ml
8.	Proteinase K	25 mg	5 x 25mg
9.	Micro Centrifuge Tubes 1.5 ml	50 tubes	250 tubes
10.	Procedure manual	1	1

Table 1.

* Contains chaotropic salt which is an irritant. Not compatible with disinfecting reagents which contain bleach. See page 3 for safety information.

† Contains sodium azide as a preservative.

Storage

-- Geno Sen's® Mini spin columns should be stored dry at room temperature (15–25°C). Storage at higher temperatures should be avoided.

-- All solutions & reagents should be stored at room temperature till the Kit is opened & unless otherwise stated. Proteinase K needs to be stored at -20°C after reconstitution.

-- Geno Sen's® Mini spin columns and all buffers and reagents can be stored under these conditions until the expiration date on the kit box without showing any deterioration in performance.

-- Lyophilized Proteinase K Can be stored at room temperature (15–25°C) until the expiration date on the kit box (Till the time it is not reconstituted).

-- Proteinase K should be reconstituted with Buffer PKB & should be aliquoted & stored at –20°C for further usage. Do not freeze–thaw the aliquots of Proteinase K more than 3 times.

Safety Information

When working with chemicals/reagents always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the material safety data sheets (MSDSs) of the respective products. These are available online in pdf format at our following websites under the download heading:

www.genomediagnostics.co.in
www.genomediagnostics.in
www.genome-diagnostics.com
www.diagnosticsgenome.com
www.moleculardiagnosticskits.com
www.realtimepcrkits.com

CAUTION: DO NOT add bleach or acidic solutions directly to waste containing Buffer MBD, Buffer MWB W1 or Buffer MWB W2.

All the three Buffers contain guanidine salts, which can form highly reactive compounds when combined with bleach. If by chance there is any spillage of these buffers, then clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of the Geno Sen's® Genomic DNA Extraction Mini Kit:

Buffer MBD:: Contains guanidine thiocyanate: harmful. Risk and safety phrases:* R20/21/22-32, S13-26-36-46

Buffer MWB W1:: Contains guanidine thiocyanate : harmful, irritant. Risk and safety phrases:* R20/21/22-32, S13-26-36-46.

Buffer MWB W2:: Contains guanidine thiocyanate : harmful, irritant. Risk and safety phrases:* R20/21/22-32, S13-26-36-46.

24-hour emergency information

Emergency medical information can be obtained 24 hours a day from:

The countries respective Poison Information Center

* R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36: Irritating to eyes and skin; S13: Keep away from food, drink and animal feeding stuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S46: If swallowed, seek medical advice immediately and show container or label.

Product Use Limitations

The Geno Sen's® Genomic DNA Extraction Mini Kit is intended for molecular biology applications. This product stand alone is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Warnings and precautions

DNA is extremely sensitive to DNases and should always be prepared with due care. Hands and dust particles may carry bacteria and molds and are the most common sources of DNase contamination. Please read General Handling & Glassware section of this manual before starting.

PCR should always be carried out using GLP. Accordingly, a PCR laboratory should always be divided into three areas: an area for preparation of reagents, an area for preparation of samples, and an area for amplification and detection. Due to the high sensitivity of PCR, it is absolutely

necessary that all reagents remain pure and uncontaminated, and should be monitored carefully and routinely. Contaminated reagents must be discarded.

Introduction

Extraction of DNA, RNA, and proteins from biological samples is a common procedure in molecular biology laboratories for analysis of the genome, transcriptome, and proteome, respectively.

DNA purified from biological material is utilized extensively for molecular biology research and is becoming an important tool in human clinical testing. Most commonly, the isolated DNA is characterized by size and quantity to provide diagnostic information about both normal and aberrant functioning of genes. For example, detection of HBV, CMV, Herpes, JC/BK Virus, MTb etc.
Typically, there are two aspects of isolating substantially un-degraded DNA from biological samples:

(a) cell lysis (or protein denaturation)
(b) DNA purification.

Several lysing reagents have been formulated to lyse cells and/or viral protein coats. A lysate is created by mixing suspended cells (or biological fluid) with the lysing reagent, or by grinding tissues with a pestle in the presence of the lysing reagent, which facilitates penetration of the lysing reagent. The lysate reagent typically contains a detergent to dissolve cells and to solubilize proteins and lipids. A strong protein denaturant (i.e., denaturing agent) is usually added to aid in inactivating RNases. In addition, a strong reductant is often included to ensure complete protein denaturation.

There are several DNA extraction kits available on the world market from different manufacturers. However the low yields are always an issue with majority of the extraction kits available on the marketplace.

Geno Sen's® Genomic DNA Extraction Mini Kit has been designed & optimized keeping in mind to achieve higher yields from the clinical samples. The comparison Data with some of the leading Brands worldwide confirms that the yields of extracted DNA obtained by Geno Sen's® Genomic DNA Extraction Mini Kit are better by about 1.0 Ct (Real TIME PCR Data) than some of the most widely sold Extraction kits worldwide.

Please study this procedure manual carefully before starting the extraction process. The process has to be followed carefully & all the safety instructions should be followed rigidly. It is also Important to note that if the instructions are not followed properly then the yields can get reduced.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

■ Ethanol (96–100%)*

- Isopropanol
- 1.5 ml microcentrifuge tubes
- Sterile, RNase-free pipet tips (pipet tips with aerosol barriers for preventing cross contamination are recommended)
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- Variable Volume autoclavable Micro-pipets.
- DNase-free Micro Centrifuge Tubes for sample processing
- Vortex
- PBS might be required for few samples.
- SputoLyse sol in case sputum samples are being processed.

Principle & Procedure

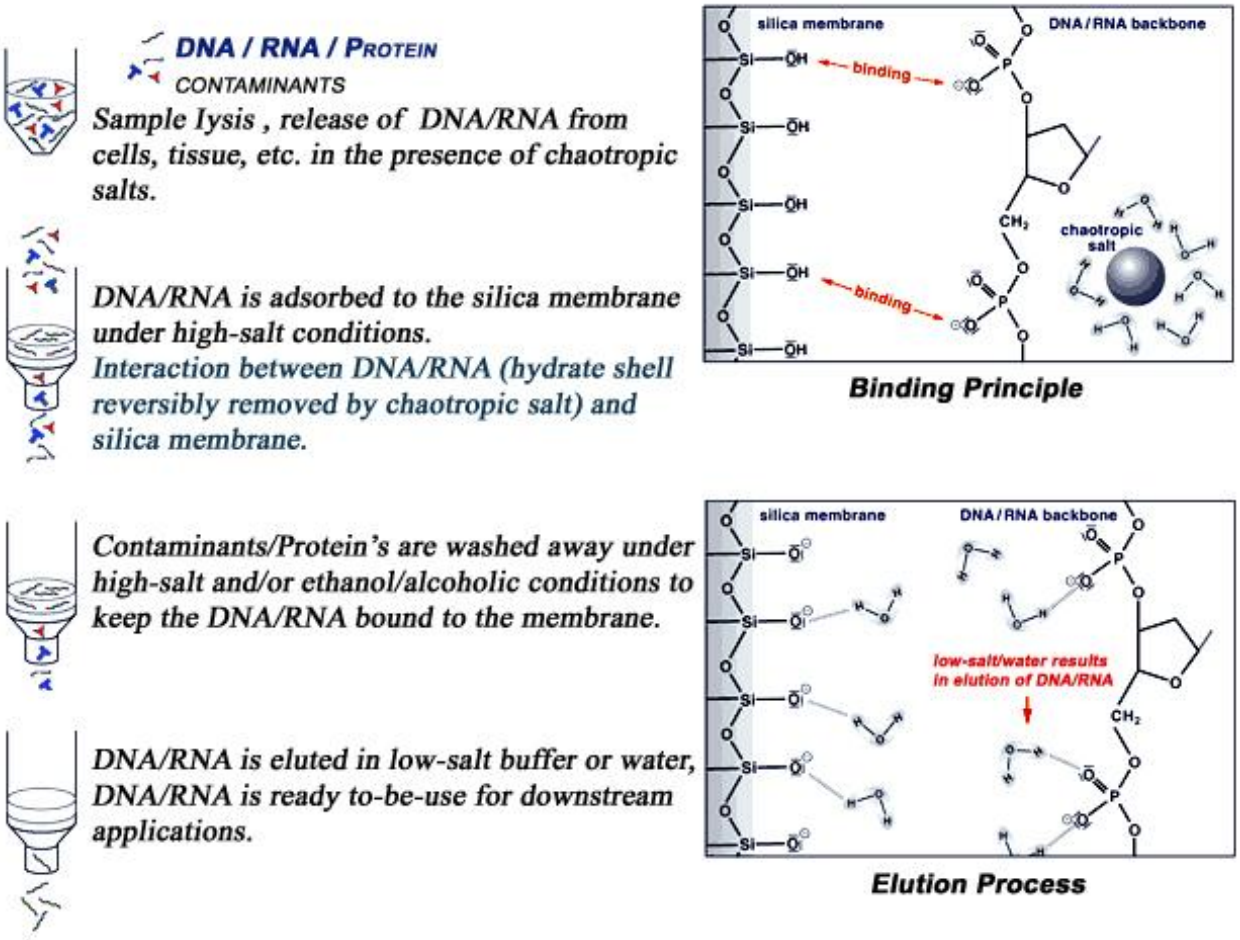
Geno Sen's® Genomic DNA Extraction Mini Kits provide's the fastest and easiest way to purify Genomic DNA for reliable use in amplification/qPCR technologies. Genomic DNA can be purified from plasma (treated with anticoagulants other than heparin), serum, body fluids, cultured cells, sputum and other body fluids. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze–thawing of plasma samples will lead to reduced DNA titers and should be avoided for optimal sensitivity. Cryo-precipitates accumulate when samples are subjected to repeated freeze–thawing cycles. This might lead to clogging of the Columns membrane resulting in reduced yields.

Geno Sen's® Genomic DNA Extraction Mini Kits are general purpose kits which can be used for isolation of Genomic DNA from a wide variety of bacteria/viruses, but performance can not be guaranteed for different specimens.

Geno Sen's® Genomic DNA extraction Mini Kits represent's a well established general-purpose technology for DNA extraction. The kit combines the selective binding properties of a silicagel- based membrane with the speed of microspin and is ideally suited for simultaneous processing of multiple samples. The sample is first lysed under highly denaturing conditions to ensure release of intact DNA. Buffering conditions are then adjusted to provide optimum binding of the DNA to the Column membrane, and the sample is loaded onto the spin column. The DNA binds to the membrane, and contaminants are efficiently washed away in two washing steps using two different wash buffers. High-quality DNA is eluted in a special buffer, ready for direct use or safe storage. The purified DNA is free of protein, nucleases, and other contaminants and inhibitors. The special membrane of the column guarantees extremely high recovery of pure, intact DNA in just twenty minutes without the use of the conventional phenol/chloroform extraction or alcohol precipitation. All buffers and reagents are guaranteed to be DNase-free.

Geno-Sen's® Genomic DNA Extraction Mini Kit.

Basic Principle of DNA/RNA extraction by Silica Columns based Isolation Method.



Adsorption to the Geno Sen's® columns membrane

The buffering conditions of the lysate are adjusted to provide optimum binding conditions for the DNA before loading the sample onto the spin column. DNA is adsorbed onto the column silica membrane during the brief centrifugation step. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit downstream enzymatic reactions, are not retained on the column membrane. If the initial sample volume is larger than 200 µl, it will be necessary to load the lysate onto the column in several steps.

Removal of residual contaminants

DNA, bound to the column membrane, is washed free of contaminants during two short centrifugation or vacuum steps. The use of two different wash buffers, MWB1 and MWB2, significantly improves the purity of the eluted DNA. Optimized wash conditions ensure complete removal of any residual contaminants without affecting DNA binding.

Elution with Buffer MEL

Buffer MEL is prepared in DNase & RNase-free water that contains sodium azide to prevent microbial growth and subsequent contamination with RNases. Sodium azide affects spectrophotometric absorbance readings between 220 and 280 nm but has no effect on downstream applications, such as PCR. Should you wish to determine the purity of the eluted DNA, elution with DNase -free water instead of Buffer MEL is recommended.

Co-purification of RNA with DNA

The Geno Sen's® Genomic DNA Extraction Mini Kit is not designed to separate RNA from the DNA, and both will be purified in parallel if present in the sample. To avoid co-purification of RNA 4 lU of (100mg/ml) RNase can be used & added to the sample before addition of Buffer MBD. RNase is not supplied with this kit & can be bought separately from several suppliers.

Since both the nucleic acids get extracted simultaneously hence please note that this Kit is only recommended for DNA extraction & is not optimized for RNA extraction as the yields for RNA will be low. For RNA extraction there is a separate optimized Kit Available. Cat. No. 98001 & 98002.

Sample volumes

Geno Sen's® spin columns can bind DNA upto 50kb in length. Actual yield will depend on sample size, sample storage, and DNA titer. The procedure is optimized for use with 200 µl samples, but samples up to 600 µl can be used. Small samples should be adjusted to 200 µl with PBS before loading, and samples with a low titer should be concentrated to 200 µl before processing if required. For samples larger than 200 µl, the amount of lysis buffer and other reagents added to the sample before loading must be increased proportionally**, but the amounts of Buffers MWB W1 and MWB W2 used in the wash steps usually do not need to be increased**. If the initial sample volume is increased, application of the lysed sample to the Mini spin column will require multiple loading steps. There is no danger of overloading the spin column, and the quality of the purified DNA will be unaffected.

Lysis

Proteinase K & The lysis Buffer MBD are provided in the kit for the Lysis of the sample. The sample is first lysed under the highly denaturing conditions provided by Buffer MBD to ensure isolation of intact DNA. If very low copy DNA is expected then Carrier DNA (NOT PROVIDED WITH THE KIT i.e. poly dA, Poly dT or poly dA:dT), can be added prior to starting the procedure to the Buffer MBD. (Conc as per the manufacturers instructions). We recommend usage of 5 µg of Carrier DNA to 200 µl of Buffer MBD. This can be done in case of Free Viral particles in Fluids or suspensions barring Urine.

Addition of internal controls

Using the Geno Sen's® Genomic DNA Extraction Mini protocols in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control DNA can be added to the lysis buffer. For optimal purification efficiency, internal control molecules should be longer than 200 nucleotides, as smaller molecules are not efficiently recovered.

Refer to the manufacturer's instructions in order to determine the optimal concentration.
Using a concentration other than that recommended may reduce amplification efficiency.

Determination of yield

Yields of DNA isolated from biological samples normally depend upon the sample used for extraction. A normal person's 200 µl whole Blood will typically provide a yield of 3-12 µg of DNA with the Geno Sen's® Genomic DNA Extraction Mini kit. While extracting from Biological samples of Free Viral particles in Fluids or suspensions barring Urine an elution with 50µl of Buffer MEL is recommended as it will ensure elution of at least 90% DNA bound to the membrane. Whereas while extracting with Cells more than 1 x 10^7/ml elution with 200 µl Buffer MEL is recommended.

Pre-Heating the Buffer MEL to 50°C before addition to the column will result in increased yields by about 5%

Important Notes

All steps of the Geno Sen's® Genomic DNA Extraction Mini protocols should be performed quickly and at room temperature.

After collection and centrifugation, plasma (untreated or treated with anticoagulants other than heparin) or serum can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –20°C to –80°C in aliquots is recommended. Frozen plasma or serum samples should not be thawed more than once. Repeated freezing and thawing leads to denaturation and precipitation of proteins, causing reduced DNA titers and subsequently reduced yields of the isolated DNA. In addition, cryoprecipitates formed by freeze–thawing will cause clogging of the column membrane.

The Geno Sen's® Genomic DNA Extraction Mini procedure is not designed to separate RNA from DNA. To avoid extraction of RNA please follow the guidelines on page 8 of this manual under the heading Co-purification of RNA & DNA.

The Geno Sen's® Genomic DNA Extraction Mini procedure isolates all DNA molecules larger than 200 nucleotides. Smaller DNA molecules will not bind quantitatively under the conditions used.

Preparation of reagents

Preparation of Proteinase K

Transfer the entire Vial of Dilution Buffer PKB i.e. Proteinase K dilution buffer (1250 **µl)** to the Vial containing the Proteinase K powder. This will lead to a final Concentration of Proteinase K to 20mg/ml.

Briefly Vortex the vial to ensure that the entire Proteinase K powder gets dissolved in the dilution buffer.

Leave it for 5 minutes at 60°C so that the Sol becomes completely transparent. Briefly Vortex the vial & then make aliquots & store at -20°C for further usage.

There are 5 vials each of Proteinase K (25mg) & PKB (Proteinase K dilution buffer provided in the 250 preps kit. Only the reconstituted vial needs to be stored at -20°C. Rest of the vials can remain at Room Temperature.

Buffer MWB W1*

Buffer MWB W1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle and in Table 2.

Buffer MWB W1 is stable for 18 months when stored closed at room temperature in dark, but only until the kit expiration date.

Table 3. Preparation of Buffer MWB W1

Kit cat. no.	No. of preps	MWB W1 concentrate	Ethanol	Final volume
98021	50	20 ml	16 ml	36 ml
98022	250	50ml/Vial	40ml/Vial	90ml/Vial

Note: There are two vials each of 50 ml provided in the 250 preps kit hence each vial should be reconstituted as per above.
The MWB W1 wash Buffer is provided extra in case extra wash steps need to be carried out.

Buffer MWB W2*†

Buffer MWB W2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffer MWB W2 concentrate as indicated on the bottle and in Table 3.
Buffer MWB W2 is stable for 18 months when stored closed at room temperature in dark, but only until
the kit expiration date.

Table 4. Preparation of Buffer MWB W2

Kit cat. no.	No. of preps	MWB W2 concentrate	Ethanol	Final volume
98021	50	12.5 ml	50 ml	62.5 ml
98022	250	25ml/Vial	100ml/Vial	125ml/Vial

Note: There are two vials each of 25 ml provided in the 250 preps kit hence each vial should be reconstituted as per above.

* Contains chaotropic salt. Take appropriate laboratory safety measures, and wear gloves when handling.
Not compatible with disinfecting agents that contain bleach. See page 6 for safety information.
† Contains sodium azide as a preservative.

Handling of Mini Spin columns

Owing to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling Mini Spin columns to avoid cross contamination between sample preparations:

■ Carefully apply the sample or solution to the Spin column. Pipet the sample into the Spin column without wetting the rim of the column.
■ Change pipet tips between all liquid transfer steps. The use of aerosol-barrier tips is recommended.
■ Avoid touching the Column membrane with the pipet tip.
■ After all pulse-vortexing steps, briefly centrifuge 1.5 ml microcentrifuge tubes to remove drops from the inside of the lid.
■ Wear gloves throughout the procedure. In case of contact between gloves and sample, change gloves immediately.

Spin protocol

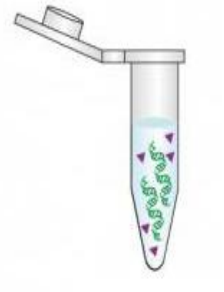
■ Close the Spin column before placing it in the microcentrifuge. Centrifuge as described in this protocol.
■ Remove the Spin column and collection tube from the micro centrifuge. Place the Spin column in a new collection tube. Discard the filtrate and the old collection tube. Please note that the filtrate may contain hazardous waste and should be disposed of properly.
■ Open only one Spin column at a time, and take care to avoid generating aerosols.
■ For efficient parallel processing of multiple samples, it is recommended to fill a rack with collection tubes to which the Spin columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the Spin columns can be placed directly in the microcentrifuge.

Centrifugation

Geno Sen's® Mini columns will fit into most standard 1.5 ml or 2 ml microcentrifuge tubes.
Adequate Collection tubes has been provided for all the steps. However if required additional 2 ml collection tubes are available separately against orders.
Centrifugation at full speed will not affect DNA yield.
Centrifugation at lower speeds should not be performed.
At the second wash step centrifugation at full speed is strongly recommended.
All centrifugation steps are carried out at room temperature.

Geno-Sen's® Genomic DNA Extraction Mini Kit.

Protocol at a glance.



Lysis Of the Sample

a) Addition of 20 µl of reconstituted Proteinase K.
b) Addition of 200 µl of sample.
c) Addition of 200 µl of Buffer MBD. Pulse Vortex for 15 secs.
d) Incubation at 60°C for 10 minutes.
e) Addition of 100 µl of Isopropanol & mixing by pulse vortexing.

Binding of the Nucleic Acids to the Silica Membrane

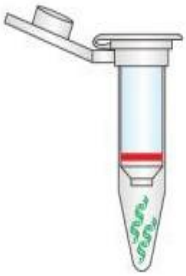
a) Loading of the above Lysate i.e 520 µl to the column.
b) Centrifuge at 12000 r.p.m. for 2 minute.
c) In case higher volumes than 200 µl of the sample are being processed the change the Collection tube & reload from the balance of the above Sol.
d) Centrifuge at 12000 r.p.m. for 2 minute.
e) Change the collection tube.

Wash Step 1 with MWB W1

a) Add 500 µl of MWB W1.
b) Centrifuge at 12000 r.p.m. for 2 minute.
c) This will remove the contaminants from the column & will leave the Nucleic acids bound to silica Membrane on the membrane.

Wash Step 2 with MWB W2

d) Add 500 µl of MWB W2.
e) Centrifuge at 12000 r.p.m. for 2 minute.
f) Centrifuge again at 14000 r.p.m. for 3 minute.
g) This will remove the contaminants from the column & will leave the Nucleic acids bound to silica Membrane on the membrane.



Elution of purified DNA/RNA

a) Add 50 µl of Buffer MEL.
b) Incubate for 1 min at Room Temperature.
c) Centrifuge at 12000 r.p.m. for 2 minutes.
d) Discard Column.

Purified DNA/RNA is now available for Down stream applications.

Geno-Sen's® Genomic DNA Extraction Mini Kit.

Detailed Spin Protocol

This protocol is for purification of Genomic DNA from 200 µl plasma, serum, urine, & other body fluids using a microcentrifuge.

Larger starting volumes, up to 600 µl (in multiples of 200 µl), can be processed by increasing the initial volumes proportionally and loading the Geno Sen's® Mini spin column multiple times, as described below in the protocol.

Important points before starting

- Read "Important Notes" (pages 9–11) before starting the protocol.
- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer MEL to room temperature for elution in step 11.
- Check that Buffer MWB W1 and Buffer MWB W2 have been prepared according to the instructions on page 10.
- Add entire Vial of Buffer PKB to Proteinase K Vial according to instructions on page 10.
- Heat a Heating Block or water Bath to 60°C

Extraction from Sputum samples.

While extracting DNA from Sputum samples it is recommended that the sputum samples should first be treated with the Geno Sen's® SputoLyse Sol (Not provided in this Kit. Need to be bought separately Cat. No. 99001 & Cat. No. 99002) to ensure the de-contamination of the samples as well as liquification of mucus & homogenization of the sample. For detailed instructions please refer to the protocol of Geno Sen's® Sputolyse Sol.

Processing un-treated samples might result in low yield or Inhibition of PCR.

Procedure for Extraction of DNA

1. Pipet 20 µl of prepared Proteinase K at the bottom of the 1.5/2.0ml microcentrifuge tube (provided).

If the sample volume is larger than 200 µl, increase the amount of Proteinase K proportionally & use larger tubes.

2. Add 200 µl of sample plasma, serum, pre-treated Sputum, body fluids, buffy coat etc. to the above microcentrifuge tube.

If the sample Volume is less than 200µl, add the rest volume of PBS to make it to 200µl.

3. Add 200 µl of Buffer MBD to the sample in the microcentrifuge tube. Mix by Pulse Vortexing for 15 secs.

To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer MBD to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.

If volumes larger than **200 µl** is being processed then the Buffer MBD should be increased accordingly in a larger tube.

Proteinase K should never be mixed with Buffer MBD & should always be dispensed separately.

4. Incubate at 60°C for 10 min in a pre-heated Heating Block or water bath.

DNA lysis is complete after 10 min at **60°C** . Longer incubation times have no effect on the yield or quality of the purified DNA.

5. Briefly centrifuge the tube to remove drops from the inside of the lid.

6. Add 100 µl of Isoproponal to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid.

If the Sample volume is greater than 200 µl, increase the amount of Isoproponal proportionally In order to ensure efficient binding, it is essential that the sample is mixed thoroughly with the Isoproponal to yield a homogeneous solution.

7. Carefully apply the entire Mix i.e. 520 µl of the solution from step 6 to the Spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 12000 rpm for 2 min. Place the Spin column into a clean 2 ml collection tube, and discard the tube containing the filtrate.

Close each spin column in order to avoid cross-contamination during centrifugation.

Centrifugation is performed at 12000 rpm in order to ensure that all the liquid passes through the column. Centrifugation at full speed will not affect the yield or purity of the DNA. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all of the solution has passed through.

8. Carefully open the Spin column, and add 500 µl of Buffer MWB W1. Close the cap, and centrifuge at 12000 rpm for 2 min. Place the Spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.

It is not necessary to increase the volume of Buffer MWB W1 even if the original sample volume was larger than 200 µl.

9. Carefully open the Spin column, and add 500 µl of Buffer MWB W2. Close the cap and centrifuge at 12000 rpm for 2 minutes. After the centrifuge has stopped Again Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

10. Place the column in a fresh collection tube (not provided) & centrifuge at full speed i.e. (20,000 x g; 14,000 rpm) for 2 min. This step is optional hence can be avoided by expert hands.

This step is carried out to eliminate any chance of possible Buffer MWB W2 carryover, perform step 10, and then continue with step 11.

Note: Residual Buffer MWB W2 in the elute may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in flow-through, containing Buffer MWB W2, contacting the Spin column. Removing the Spin column and collection tube from the rotor may also cause flow-through to come into contact with the Spin column. In these cases, the optional step 10 should be performed.

11. Place the Spin column in a clean (RNaes & DNaes free)1.5/2.0 ml microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the Spin column and add 50 µl of Buffer MEL equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 12000 rpm for 2 min.

A single elution with 50 µl of Buffer MEL is sufficient to elute at least 90% of the DNA from the Spin column. Performing a double elution using 2 x 40 µl of Buffer MEL might increase yield by up to 5%-7%. Elution with volumes of less than 30 µl will lead to reduced yields and will not increase the final concentration of DNA in the elute. Usage of Pre-Heated MEL buffer to 50°C might increase the yield by 5%-7%.

Use the DNA Directly or store at –20°C or –70°C for further usage.

When larger yields of DNA are expected then it is recommended that the elution be carried out in **200 µl of Buffer MEL .** This will help increase the yield of DNA greatly. However for clinical samples elution in **50 µl of Buffer MEL** is highly recommended.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise

		Comments and suggestions
Little or no DNA in the elute		
a)	Very Low Conc. Of cells	Concentrate the sample by any conc. Method described in several protocols. In case of Whole Blood, prepare a Buffy Coat.
b)	Forgot to add reconstituted Proteinase K	Repeat the extraction process with a new sample & ensure that Proteinase K is dispensed in the tube before the sample.
c)	Reconstituted Proteinase K	Repeat the extraction process with a new sample & ensure

	added to Buffer MBD.	that Proteinase K is dispensed in the tube before the sample & only then the Buffer MBD is added to Mix.
d)	Improper cell Lysis due to reduced activity of Proteinase K.	Repeat the extraction process with a new sample & ensure that a New Proteinase K Vial is reconstituted before beginning the extraction process.
e)	Improper cell Lysis due to inadequate mixing with Buffer MBD	Repeat the extraction process with a new sample & ensure that Mixing & Vortexing with Buffer MBD is done thorougly.
f)	Sample frozen and thawed more than once	Repeated freezing and thawing should be avoided. Always use fresh samples or samples thawed only once.
g)	Inefficient protein denaturation in Buffer MBD	Precipitate, formed in Buffer MBD. Redissolve the precipitate by heating at 80°C. and repeat the procedure with a new sample.
h)	No isopropanol added to the lysate (step 6)	Repeat the purification procedure with a new sample. & ensure that Isoproponal is added at Step 6.
I)	Ethanol used at Step 6 Instead of Isoproponal.	Repeat the purification procedure with a new sample. Use only Isoproponal at Step 6.
j)	Incubation Carried out at RT instead of 60°C	Repeat the purification procedure with a new sample. Enusre that 10 min. incubation is done at 60°C.
k)	No ethanol added to MWB W1 & MWB W2.	Repeat the purification procedure with a new sample. Ensure that MWB W1 & MWB W2 as per the details Provided in Preparation of reagents Table 3 & 4.
l)	Isoproponal used Instead of Ethanol In Wash Buffers.	Repeat the purification procedure with a new sample. Ensure that only Ethanol is added to MWB W1 & MWB W2.
m)	Buffer MWB W1 or MWB W2 prepared incorrectly	Check that Buffer MWB W1 and MWB W2 concentrates were diluted with correct volumes of pure (96–100%) ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with a new sample. Also ensure that 70% ethanol is not used.
n)	Buffers MWB W1 and MWB W2 used in the wrong order	Ensure that Buffer MWB W1 and Buffer MWB W2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
k)	DNase contamination in Buffer MEL	Discard contaminated Buffer MEL. Repeat the purification procedure with a new sample and a fresh tube of Buffer MEL.

DNA does not perform well in subsequent enzymatic reactions

a)	Little or no DNA in the	Check "Little or no DNA in the elute," above, for

	elute	possible reasons.
b)	High level of residual RNA.	Use the optional RNase step in the protocol.
c)	Reduced sensitivity	Adjust the volume of MEL buffer for elution. Determine the maximum volume of elute suitable for your RT-PCR. Reduce the volume of elute added to the RT-PCR.
d)	New combination of reverse transcriptase and Taq DNA polymerase used	If enzymes are changed, it may be necessary to readjust the amount of carrier RNA solution added to Buffer MBR.
e)	Check the Rotor of Centrifuge for vibration.	Change the Centrifuge & perform the extraction again as their could be possibility of MBW W2 being eluted alongwith the DNA.

General handling

a)	Lysate not completely passed through the membrane	Using spin protocol: Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane.
b)	Clogged membrane	Cryoprecipitates have formed in plasma due to repeated freezing and thawing. Do not use plasma that has been frozen and thawed more than once.
c)	Cross-contamination	To avoid cross-contamination when handling Geno Sen's® between samples Mini spin columns follow the guidelines in "Handling of Geno Sen's® Mini columns" on page 11. Repeat the purification procedure with new samples.

General handling

Proper microbiological, aseptic technique should always be used when working with DNA. Hands and dust particles may carry bacteria and molds and are the most common sources of DNase contamination. Always wear latex or vinyl gloves while handling reagents and DNA samples to prevent DNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. During the procedure, work quickly to avoid degradation of DNA by endogenous or residual DNases.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally DNase-free and do not require pretreatment to inactivate DNases.

Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is DNase free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by DNase-free water (see "Solutions", page 37). Alternatively, chloroform resistant plasticware can be rinsed with chloroform* to inactivate DNases.

Glassware

Glassware should be treated before use to ensure that it is DNase-free. Glassware used for DNA work should be cleaned with detergent,* thoroughly rinsed, and oven baked at >240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many DNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Rinse the glassware with 0.1% DEPC (0.1% in water) overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to remove residual DEPC.

DEPC is a strong, but not absolute, inhibitor of DNases. It is commonly used at a concentration of 0.1% to inactivate DNases on glass or plasticware or to create DNase-free solutions and water. DEPC inactivates DNases by covalent modification. Trace amounts of DEPC will modify purine residues in DNA by carbethoxylation. Carbethoxylated DNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be removed from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Add 0.1 ml DEPC to 100 ml of the solution to be treated. Shake vigorously to bring the DEPC into solution, or let the solution bake for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. It may be desirable to test water sources for the presence of contaminating DNases since many sources of distilled water are free of DNase activity.

Note: Geno Sen's® Genomic DNA extraction kit buffers are not rendered DNase-free by DEPC treatment and are therefore free of any DEPC contamination.

Product Warranty and Satisfaction Guarantee

GENOME DIAGNOSTICS PVT. LTD. guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, GENOME DIAGNOSTICS PVT. LTD. will replace it free of charge. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a GENOME DIAGNOSTICS PVT. LTD. product does not meet your expectations, simply call your local distributor. We will replace or exchange the product — as you wish. Separate conditions apply to GENOME DIAGNOSTICS PVT. LTD. scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of GENOME DIAGNOSTICS PVT. LTD. terms and conditions can be obtained on request. If you have questions about product specifications or performance, please call GENOME DIAGNOSTICS PVT. LTD or email us .

Technical Assistance

If you have any questions or experience any difficulties regarding the Geno Sen's® Genomic DNA Mini Kit or GENOME DIAGNOSTICS PVT. LTD. products in general, please do not hesitate to contact us.

For technical assistance and more information, please email our Technical Support center at

www.genomediagnostics.co.in
www.genomediagnostics.in
www.genome-diagnostics.com
www.diagnosticsgenome.com
www.moleculardiagnosticskits.com
www.realtimepcrkits.com

Quality Control

In accordance with Genome Diagnostics Pvt. Ltd. ISO 13485-certified Quality Management System, each lot of Geno Sen's® Genomic DNA Extraction Mini Kits is tested against predetermined specifications to ensure consistent product quality.

Ordering Information

Geno Sen's® Genomic DNA Extraction Mini Kit — for DNA purification from Plasma, serum, Buffy Coat and cell-free body fluids like C.S.F. Urine etc.			
S.No.	**Cat. No.**	**Preps**	**Product**
1.	**98021**	**50**	**Geno Sen's® Genomic DNA Extraction Mini Kit**
2.	**98022**	**250**	**Geno Sen's® Genomic DNA Extraction Mini Kit**

Geno Sen's Viral RNA extraction Mini Kit — for Viral RNA purification from serum, plasma and body fluids, cell culture supernants, C.S.F.			
S.No.	**Cat. No.**	**Preps**	**Product**
1.	**98001**	**50**	**Geno Sen's® Viral RNA extraction Mini Kit**
2.	**98002**	**250**	**Geno Sen's® Viral RNA Extraction Mini Kit**

Geno-Sen's® Genomic DNA Extraction Mini Kit.

Additional Reagents which might be required for extraction.			
S.No.	**Cat. No.**	**Qty**	**Product**
1.	99001	100 ml.	Geno Sen's® SputoLyse Solution for de-contamination of sputum samples before extracting DNA.
2.	99002	500 ml.	Geno Sen's® SputoLyse Solution for de-contamination of sputum samples before extracting DNA.
3.	99006	350µg	Geno Sen's®Carrier RNA for RNA extraction.
4.	99007	1mg	Geno Sen's®Carrier RNA for RNA extraction.
5.	99008	50mg	Geno Sen's®Carrier RNA for RNA extraction.
6.	99011		Geno Sen's®DNase
7.	99012		Geno Sen's®DNase
8.	99016		Geno Sen's®RNase
9.	99017		Geno Sen's®RNase
10.	99021	100 ml	PBS 1 X for compensating volumes or for additional washing step in pre-treatment of samples.
11.	99022	500 ml.	PBS 1 X for compensating volumes or for additional washing step in pre-treatment of samples.
12.	99051	Pack of 1000 tubes	Collection Tubes for the columns.