Geno-Sen's® Viral RNA Extraction Mini Kit.

Geno-Sen's®

Viral RNA Extraction Mini Kit

ICMR Validated

For purification of viral RNA from

Serum
plasma
Cell-free body fluids
C.S.F
Cell-culture supernatants

Procedure Manual

Since 2015
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(An ISO 13485:2016 Certified Company)

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Contents

Geno-Sen's® Viral RNA Extraction Mini Kit.

Kit Contents: (Geno Sen's® Viral RNA Extraction Mini Kit)

S.No.	Contents	REF 98001 50 Preps	REF 98003 100 Preps	REF 98002 250 Preps
1.	Spin Columns	**50**	**100**	**250**
2.	Collection Tubes (2 ml) Including the one's fitted in the columns.	100+50 fitted With columns	200+100 fitted With columns	500+250 Fitted with columns
3.	Binding Buffer MBR*	35 ml	70 ml	2 x 87 ml
4.	Wash Buffer MWB W1* (concentrate)	20 ml	40 ml	2 x 50 ml
5.	Wash Buffer MWB W2*† (concentrate)	12.5 ml	25 ml	2 x 25 ml
6.	Elution Buffer MEL†	10 ml	20 ml	50 ml
7.	Carrier RNA (poly A)	350 µg	2 x 350 µg	5 x 350 µg
8.	Micro Centrifuge Tubes	50 tubes	100 tubes	250 tubes
9.	Procedure manual	1	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 (15–30°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. Carrier RNA 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 carrier RNA can be stored at room temperature (15–30°C) until the expiration date on the kit box (Till the time it is not reconstituted).
-- Carrier RNA should be reconstituted with Buffer MEL & should be aliquoted & stored at –20°C for further usage. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Reconstituted Carrier RNA should be mixed with buffer MBR as described on page 10-11 immediately before starting the extraction process . This solution should always be prepared fresh & should not be stored for future use as stored solution can result in lower yields.

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
CAUTION: DO NOT add bleach or acidic solutions directly to waste containing

Buffer MBR, 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® Viral RNA Extraction Mini Kit:

Buffer MBR:: 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® Viral RNA 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 to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Warnings and precautions

RNA is extremely sensitive to RNases and should always be prepared with due care. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Please read —Handling RNAll in the Appendix (page 19) 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.

RNA 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 RNA is characterized by size and quantity to provide diagnostic information about both normal and aberrant functioning of genes. For example, detection & quantitation of HCV, HIV, HEV, JEV etc.

Typically, there are three aspects of isolating substantially Intact RNA from biological samples:

(1) The cells or viral protein coats are lysed to release RNA;

(2) Ribonucleases (RNases) are inactivated to prevent RNA degradation;

(3) Contaminants are removed to purify the preparation. Because of the abundance and stability of RNases in biological materials, it is important that cell or protein coat lysis and RNase inactivation be substantially simultaneous. Therefore, in its simplest form, the isolation of RNA is reduced to just two main steps:

(a) Cell lysis (or protein denaturation)/RNase inactivation;
(b) RNA purification.

Several lysing reagents/chemicals have been formulated to lyse cells and/or viral protein coats and inactivate RNases substantially simultaneously. 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 RNA extraction kits available on the world market from different manufacturers.

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.

Geno-Sen's® Viral RNA Extraction Mini Kit.

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%)*
- 1.5 ml microcentrifuge tubes
- Sterile, RNase-free pipette tips (pipette 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-pipettes.
- RNase-free Micro Centrifuge Tubes for sample processing
- Vortex
- PBS might be required for few samples.

Principle & Procedure

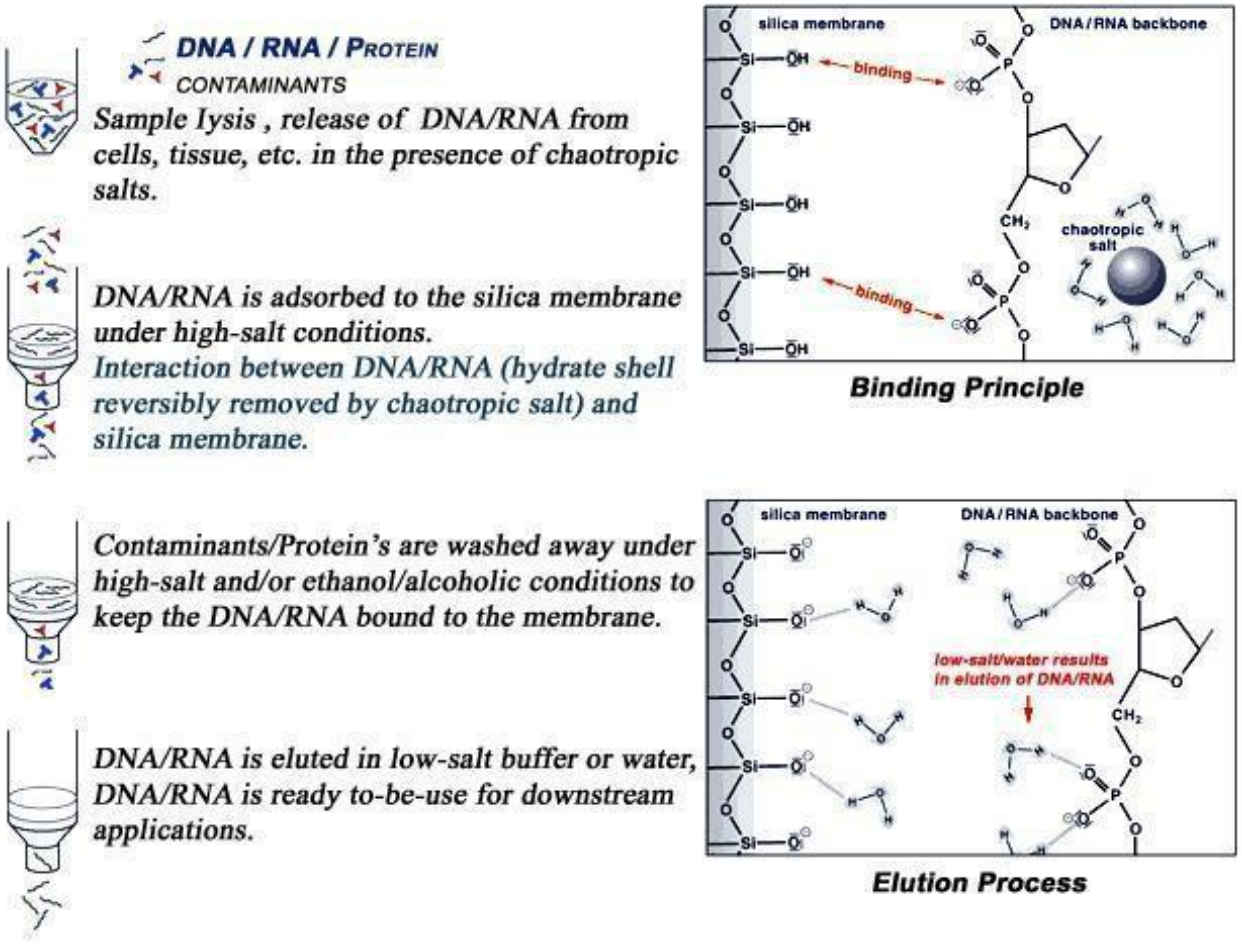
Geno Sen's® Viral RNA extraction Mini Kits provide's the fastest and easiest way to purify viral RNA for reliable use in amplification/qPCR technologies. Viral RNA can be purified from plasma (treated with anticoagulants other than heparin), serum, and other cell-free body fluids. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze–thawing of plasma samples will lead to reduced viral 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® Viral RNA extraction Mini Kits are general purpose kits which can be used for isolation of viral RNA from a wide variety of viruses, but performance cannot be guaranteed for every virus.

Geno Sen's® Viral RNA extraction Mini Kits represent's a well established general-purpose technology for viral RNA preparation. 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 inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the Column membrane, and the sample is loaded onto the spin column. The RNA binds to the membrane, and contaminants are efficiently washed away in two washing steps using two different wash buffers. High-quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors. The special membrane of the column guarantees extremely high recovery of pure, intact RNA in just twenty minutes without the use of the conventional phenol/chloroform extraction or alcohol precipitation. All buffers and reagents are guaranteed to be RNase-free.

Geno-Sen's® Viral RNA 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 viral RNA before loading the sample onto the spin column. Viral RNA is adsorbed onto the column silica membrane during two brief centrifugation steps. 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 150 µl, it will be necessary to load the lysate onto the column in several steps.

Removal of residual contaminants

Viral RNA, bound to the column membrane, is washed free of contaminants during two short centrifugation steps. The use of two different wash buffers, MWB W1 and MWB W2, significantly improve the purity of the eluted RNA. Optimized wash conditions ensure complete removal of any residual contaminants without affecting RNA binding.

Elution with Buffer MEL

Buffer MEL is 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 RT-PCR. Should you wish to determine the purity of the eluted RNA, elution with RNase-free water instead of Buffer MEL is recommended.

Cellular DNA contamination

The Geno Sen's® Viral RNA extraction Mini Kit is not designed to separate viral RNA from cellular DNA, and both will be purified in parallel if present in the sample. To avoid co-purification of cellular DNA, the use of cell-free body fluids for preparation of viral RNA is recommended. Samples containing cells, such as cerebrospinal fluid, bone marrow, urine, and most swabs, should first be filtered, or centrifuged for 10 minutes at 1500 x g and the supernatant used. If RNA and DNA have been isolated in parallel, the elute can be DNase digested using RNase-free DNase, followed by heat treatment (15 min, 70°C) to inactivate the DNase.

Since both the nucleic acids get extracted simultaneously hence please note that this Kit is only recommended for VIRAL RNA extraction & is optimized for VIRAL RNA extraction & not for DNA extraction as the yields for DNA might be low. For DNA extraction there is a separate optimized Kit Available. Cat. No. 98021 & 98022& Cat No 98023

Sample volumes

Geno Sen's® spin columns can bind RNA greater than 200 nucleotides in length. Actual yield will depend on sample size, sample storage, and virus titer. The procedure is optimized for use with 150 µl samples, but samples up to 600 µl can be used. Small samples should be adjusted to 150 µl with PBS before loading, and samples with a low viral titer should be concentrated to 150 µl before processing if required. For samples larger than 150 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 RNA will not be affected.

Lysis

The sample is first lysed under the highly denaturing conditions provided by Buffer MBR to inactivate RNases and to ensure isolation of intact viral RNA. Carrier RNA, added prior to starting the procedure to Buffer MBR, improves the binding of viral RNA to the columns membrane especially in case of low-titer samples, and limits possible degradation of the viral RNA due to any residual RNase activity.

Carrier RNA

Carrier RNA serves two purposes. Firstly, it enhances binding of viral nucleic acids to the spin column membrane, even if there are very few target molecules in the sample. Secondly, the addition of large amounts of carrier RNA reduces the chance of viral RNA degradation in the rare event that RNase molecules escape denaturation by the chaotropic salts and detergent in Buffer MBR. If carrier RNA is not added to Buffer MBR this may lead to reduced viral RNA recovery.

The amount of lyophilized carrier RNA provided is sufficient for the volume of Buffer MBR supplied with the kit. The concentration of carrier RNA has been adjusted so that the Geno Sen's® Viral RNA extraction Mini Kit can be used as a generic purification system compatible with many different amplification systems and is suitable for a wide range of RNA viruses.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Elutes from this kit contain both viral nucleic acids and carrier RNA, and amounts of carrier RNA will greatly exceed amounts of viral nucleic acids. Calculations of how much elute to add to downstream amplifications should therefore be based on the amount of carrier RNA added. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to Buffer MBR in case the yields are low with the recommended volumes.

Addition of internal controls

Using the Geno Sen's® Viral RNA extraction Mini protocols in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control RNA or DNA can be added together with the carrier RNA 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 viral RNA isolated from biological samples are normally less than 1 µg and therefore difficult to determine photometrically. Keep in mind that the carrier RNA (6.0 µg per 150 µl sample) will account for most of the RNA present. Quantitative RT-PCR is recommended for determination of viral RNA yield.

Determination of viral RNA length

The size distribution of viral RNA purified using Geno Sen's® spin columns can be checked by denaturing agarose gel electrophoresis followed by hybridization with a virus specific labeled probe and autoradiography (Sambrook, J. and Russell, D. W. [2001] Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

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Important Notes

If preparing RNA for the first time please read —Handling RNA— in the Appendix of this manual (page 19). All steps of the Geno Sen's® Viral RNA 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 viral titers and subsequently reduced yields of the isolated viral RNA. In addition, cryoprecipitates formed by freeze–thawing will cause clogging of the column membrane. If cryoprecipitates are visible, they can be pelleted by briefly centrifuging at 6800 x g for 3 minutes. The cleared supernatant should be removed, without disturbing the pellet, and processed immediately. This step will not reduce viral titers.

The Geno Sen's® Viral RNA extraction Mini procedure is not designed to separate RNA from DNA. To avoid cellular DNA contamination follow the guidelines in Cellular DNA contamination on page 8 of this manual.

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

Preparation of reagents

Addition of carrier RNA to Buffer MBR*

Add 350 µl Buffer MEL to the tube containing 350 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Check Buffer MBR for precipitate, and if necessary incubate at 80°C until the precipitate is dissolved. Calculate the volume of Buffer MBR–carrier RNA mix needed per batch of samples by selecting the number of samples to be **simultaneously** processed from Table 2. For larger numbers of samples, volumes can be calculated using the following sample calculation:

Buffer MBR*: **n** x 0.6 ml = **x** ml
Carrier RNA dissolved in Buffer MEL:: n x 6 µl = **y** µl

* 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.

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where: **n** = number of samples to be processed simultaneously **x**
= calculated volume of Buffer MBR (in ml)
y = volume of carrier RNA–Buffer MEL to add to Buffer MBR (in µl)

Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Table 2. Volumes of Buffer MBR and carrier RNA–Buffer MEL mix required for the Geno Sen's® Viral RNA extraction Mini procedure

No. Samples	Vol. Buffer MBR (ml)	Vol. carrier RNA–MEL (µl)
1	**0.6**	**6**
5	**3.0**	30
10	**6.0**	**60**
20	**12.0**	**120**
30	**18.0**	**180**
40	24.0	240
50	30.0	300

Note: The sample-preparation procedure is optimized for 6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing Buffer MBR. (Use of less than or higher than 6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.)

Mix of Buffer MBR & carrier RNA should always be prepared fresh to get the best results

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 3.

Buffer MWB W1 is stable for 24 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
98001	50	20 ml	16 ml	36 ml
98003	100	40ml	32 ml	72ml
98002	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 4.

Buffer MWB W2 is stable for 24 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
98001	50	12.5 ml	50 ml	62.5 ml
98003	100	25ml	100ml	125 ml
98002	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 pipette tips between all liquid transfer steps. The use of aerosol-barrier tips is recommended.
- Avoid touching the Column membrane with the pipette tip.
- After all pulse-vortexing steps, briefly centrifuge 1.5 ml micro centrifuge 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

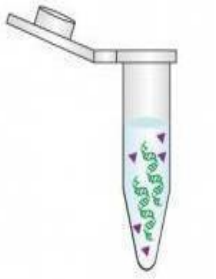
- Close the Spin column before placing it in the microcentrifuge. Centrifuge as described in this protocol.
- Remove the Spin column and collection tube from the micro centrifuge. Place the Spin column in a new collection tube. Discard the filtrate and the old collection tube. Please note that the filtrate 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 additional 2 ml collection tubes are available separately.
Centrifugation at full speed will not affect RNA 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® Viral RNA Extraction Mini Kit.

Protocol at a glance.



Lysis Of the Sample

a) Addition of 600 µl of Buffer MBR Mixed with Carrier RNA + 150 µl of sample.
b) Incubation at Room Temperature for 10 minutes.
c) Addition of 600 µl of ethanol & mixing by pulse vortexing.

Binding of the Nucleic Acids to the Silica Membrane

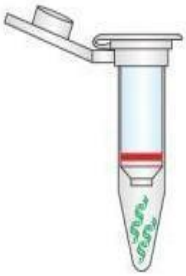
a) Loading of the above lysate i.e 675 µl to the column.
b) Centrifuge at 12000r.p.m. for 2 minute.
c) Discard the supernatant & reload the balance 675 µl Of the above Sol.
d) Centrifuge at 12000 r.p.m. for 2 minute.
e) Change the collection tube.

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) Discard supernatant and add 300 µl of MWB W2.
g) Centrifuge at 12000 r.p.m. for 2 minute.
h) Centrifuge again at 14000 r.p.m. for 3 minute.
i) This will remove the contaminants from the column & will leave the Nucleic acids bound to silica Membrane on the membrane.



Elution of purified RNA/DNA

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 RNA/DNA is now available for Down stream applications.

Geno-Sen's® Viral RNA Extraction Mini Kit.

Detailed Spin Protocol

This protocol is for purification of viral RNA from 150 µl plasma, serum, urine, cell culture media, C.S.F or cell-free body fluids using a microcentrifuge.

Larger starting volumes, up to 600 µl (in multiples of 150 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. Some samples with very low viral titers should be concentrated before the purification procedure; see ‖Protocol: Sample Concentration— (page 16).

Important points before starting

- Read —Important Notes‖ (page 10) before starting the protocol.
- All centrifugation steps are carried out at room temperature (15–30°C).

Things to do before starting

- Equilibrate samples to room temperature (15–30°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 11.
- Add carrier RNA reconstituted in Buffer MEL to Buffer MBR according to instructions on page 10-11.

Procedure

1. Pipet 600 µl of prepared Buffer MBR containing carrier RNA into a 1.5/2.0ml microcentrifuge tube.

If the sample volume is larger than 150 µl, increase the amount of Buffer MBR–carrier RNA proportionally & use larger tubes.

2. Add 150 µl plasma, serum, urine, cell-culture supernatant, C.S.F or cell-free body fluid to the Buffer MBR–carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 secs.

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

3. Incubate at room temperature (15–30°C) for 10 min.

Viral particle lysis is complete after 10 min at room temperature. Longer incubation times have no effect on the yield or quality of the purified RNA. Potentially infectious agents and RNases are inactivated in Buffer MBR.

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

5. Add 600 µl of ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid.

Only highly purified ethanol should be used since other alcohols may result in reduced RNA yield and purity.

Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. If the Sample volume is greater than 150 µl, increase the amount of ethanol proportionally In order to ensure efficient binding, it is essential that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution.

6. Carefully apply 675 µl of the solution from step 5 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. Discard the supernatant 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 viral RNA. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all of the solution has passed through.

7. Carefully open the Spin column, and repeat step 6.

If the sample volume was greater than 150 µl, repeat this step until all of the lysate has been loaded onto the spin column.

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 150 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.

10. Carefully open the Spin column, discard the supernatant. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

11. 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.

12. Place the Spin column in a clean (RNaes & DNaes free)1.5/2.0 ml micro centrifuge 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 RNA 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 RNA in the elute. Usage of Pre-Heated MEL buffer to 50°C might increase the yield by 5%-7%.

Viral RNA is stable for up to one year when stored at –20°C or –70°C.

Protocol: Sample Concentration

Plasma, serum, urine, cerebrospinal fluid, bone marrow, and other body fluids often have very low viral titers. In these cases, concentrating samples of up to 3.5 ml to a final volume of 150 µl is recommended.

Important point before starting

■ Use centrifugal microconcentrators such as Centricon®-100 (Amicon: 2 ml, cat. no. 4211), Microsep 100 (Filtron: 3.5 ml, cat. no. OD100C40), Ultrafree®-CL (Millipore: 2 ml, cat. no. UFC4 THK 25), or equivalent from other suppliers.

Procedure

- **Apply up to 3.5 ml of sample to the micro-concentrator following the manufacturer's instructions.**
- **Centrifuge according to manufacturer's instructions to a final volume of 150 µl.**

  Some samples, plasma in particular, may be difficult to concentrate to 150 µl due to high viscosity. Centrifugation for up to 6 hours may be necessary.
- **Pipet 150 µl of concentrated sample into a 1.5 ml microcentrifuge tube, and follow the Geno Sen's® Viral RNA Mini Spin Protocol on page 14.**

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Pipet 150 µl of concentrated sample into a 1.5 ml microcentrifuge tube, and follow the Geno Sen's® Viral RNA Mini Spin Protocol on page 14.

Troubleshooting

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

Comments and suggestions

Little or no RNA in the elute

a)	Carrier RNA not added to Buffer MBR	Reconstitute carrier RNA in Buffer MEL and mix with Buffer MBR as described on page 10-11. Repeat the purification procedure with new samples
b)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer MEL was not stored at –20°C or underwent multiple freeze–thaw cycles. Alternatively, Buffer MBR–carrier RNA mixture was stored & used. **Always use Freshly prepared MIX.**
c)	Sample frozen and thawed more than once	Repeated freezing and thawing should be avoided. Always use fresh samples or samples thawed only once.
d)	Low concentration of virus in the sample	Concentrate the sample volume to 140 µl using a microconcentrator. Repeat the RNA purification procedure with a new sample. See —Protocol: Sample Concentrationll on page 16.
e)	Inefficient protein denaturation in Buffer MBR	Precipitate, formed in Buffer MBR–carrier RNA after mixing. Redissolve the precipitate by heating at 80°C. and repeat the procedure with a new sample.
f)	Buffer MBR prepared incorrectly	Check Buffer MBR for precipitate. Dissolve precipitate by incubation at 80°C.
g)	No ethanol added to the lysate (step 5)	Repeat the purification procedure with a new sample.
h)	Low percentage ethanol used	Repeat the purification procedure with a new sample. Use 96–100% ethanol in step 5. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
i)	Isopropanol used	We strictly recommend the use of ethanol as

	instead of ethanol	isopropanol causes reduced yields.
j)	RNA degraded	Often RNA is degraded by RNases in the starting material (plasma, serum, body fluids). Ensure that the samples are processed quickly. If necessary, add RNase inhibitor to the sample. Check for RNase contamination of buffers and water, and ensure that no RNase is introduced during the procedure.
k)	RNase contamination in Buffer MEL	Discard contaminated Buffer MEL. Repeat the purification procedure with a new sample and a fresh tube of Buffer MEL.
l)	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.
m)	Buffer MWB W1 or MWB W2 prepared with 70% ethanol	Check that Buffer MWB W1 and MWB W2 concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample.
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.

RNA does not perform well in subsequent enzymatic reactions

a)	Little or no RNA in the elute	Check ―Little or no RNA in the elute,‖ above, for possible reasons.
b)	Inefficient virus lysis in Buffer MBR	Precipitate formed in Buffer MBR–carrier RNA due to temperature change before start of process. Repeat the procedure with new samples, and ensure that no precipitate has formed in Buffer MBR–carrier RNA at the beginning of the process.
c)	Buffer MBR prepared incorrectly	Ensure that carrier RNA has been reconstituted in Buffer MEL and added to Buffer MBR (see page 10-11).
d)	Too much carrier in the elute	RNA Determine the maximum amount of carrier RNA suitable for your RT-PCR. Adjust the concentration of carrier RNA added to Buffer MBR accordingly.
e)	Reduced sensitivity	Determine the maximum volume of elute suitable for your RT-PCR. Reduce the volume of elute added to the RT-PCR.
f)	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.
g)	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.

DNA contamination

	DNA and RNA present in the sample	To avoid co purification of DNA, use of cell-free body fluids for preparation of viral RNA is recommended. Samples containing cells, such as cerebrospinal fluid, bone marrow, urine, and most swabs, should be made cell-free by centrifugation or filtration. If using centrifugation, pellet the cells for 10 min at 1500 x g

	and use supernatant for isolation of viral RNA. If DNAfree RNA is required, digest either the sample or the elute with RNase-free DNase. DNase in the elute must be inactivated by heat treatment (15 min, 70°C).

General handling

a) Lysate not completely passed through the	Using spin protocol: Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane. 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 kit between samples Mini spin columns follow the guidelines in —Handling of Geno Sen's® Mini columnsll on page 12. Repeat the purification procedure with new samples.

Appendix

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase 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 RNA by endogenous or residual RNases.

Disposable plasticware

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

Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNasefree. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water. Alternatively, chloroformresistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA 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 RNases. Oven baking will inactivate ribonucleases. 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.

Note: Corex® tubes should be rendered RNase-free by treatment with DEPC and not by baking. This will reduce the failure rate of this type of tube during centrifugation.

DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA 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 RNases since many sources of distilled water are free of RNase activity.

Note: Geno Sen's® Viral RNA buffers are not rendered RNase-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

Geno-Sen's® Viral RNA Extraction Mini Kit.

If you have any questions or experience any difficulties regarding the Geno Sen's® Viral RNA 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

Quality Control

In accordance with Genome Diagnostics Pvt. Ltd. ISO 13485-certified Quality Management System, each lot of Geno Sen's® Viral RNA 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.	98023	100	Geno Sen's® Viral DNA extraction Mini Kit
3.	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.	98003	100	Geno Sen's® Viral RNA extraction Mini Kit
3.	98002	250	Geno Sen's® Viral RNA 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.