Geno-Sen's®

Soft & Hard Tissue RNA Extraction Mini Kit

For purification of Tissue RNA from

Soft & Hard Tissue Samples e.g

Liver
Spleen
Thymus
Heart
Kidney
Brain etc.

Procedure Manual

July 2012

Genome Diagnostics Pvt. Ltd.

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

Genome Diagnostics Pvt. Ltd.,
Up Mohal Naryal, Khasra No. 427,
Opp. Divya Packers, Old Timber Depot Road,
Near Sector 4, Parwanoo. Dist Solan H.P.
Email: genome24@rediffmail.com
Tel: 01792-234285 Fax: 01792-234286

EC REP
EMERGO EUROPE
MolenStraat 15, 2513 BH, The Hague
The Netherlands
Phone: +31.70.345.8570 Fax: +31.70.346.7299

Contents

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

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

S.No	Contents	REF 98004 50 preps	REF 98005 250 preps
1.	Spin Columns	**50**	**250**
2.	Collection Tubes (2 ml) Including the one's fitted in the columns	150+50 fitted with columns	750+250 fitted with columns
3.	Tissue Lysis Buffer TLR	8 ml	40 ml
4.	Binding Buffer MBTR*	12 ml	60 ml
5.	Wash Buffer MWB W1* (concentrate)	20 ml	2 x 50 ml
6.	Wash Buffer MWB W2*† (concentrate)	12.5 ml	2 x 25 ml
7.	Elution Buffer MEL†	10 ml	50 ml
8.	Carrier RNA (poly A)	350 µg	5 x 350 µg
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. Carrier RNA (poly A) 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 (poly A)Can be stored at room temperature (15–25°C) until the expiration date on the kit box (Till the time it is not reconstituted).

-- Carrier RNA (poly A) 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 (poly A) 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 MBTD, Buffer MWB W1 or Buffer MWB W2.

All the three Buffers contain guanidine salts except for Tissue Lysis Buffer (TLR), 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® Tissue RNA Extraction Mini Kit:

Buffer MBTR:: 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® Tissue 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 RNA 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 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 RNA, DNA, 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 of DENGUE, HIV, Cancer, HCV, etc.

Typically, there are two aspects of isolating substantially un-degraded RNA from Tissue samples:

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

Several lysing reagents have been formulated to lyse cells and/or viral protein coats. A lysate is created by mixing suspended Tissues/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 Tissue RNA 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® Tissue RNA 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 RNA obtained by Geno Sen's® Tissue RNA Extraction Mini Kit are better by about 1.0-2.0 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.
- RNAse-free Micro Centrifuge Tubes for sample processing
- Vortex
- 1X PBS

Principle & Procedure

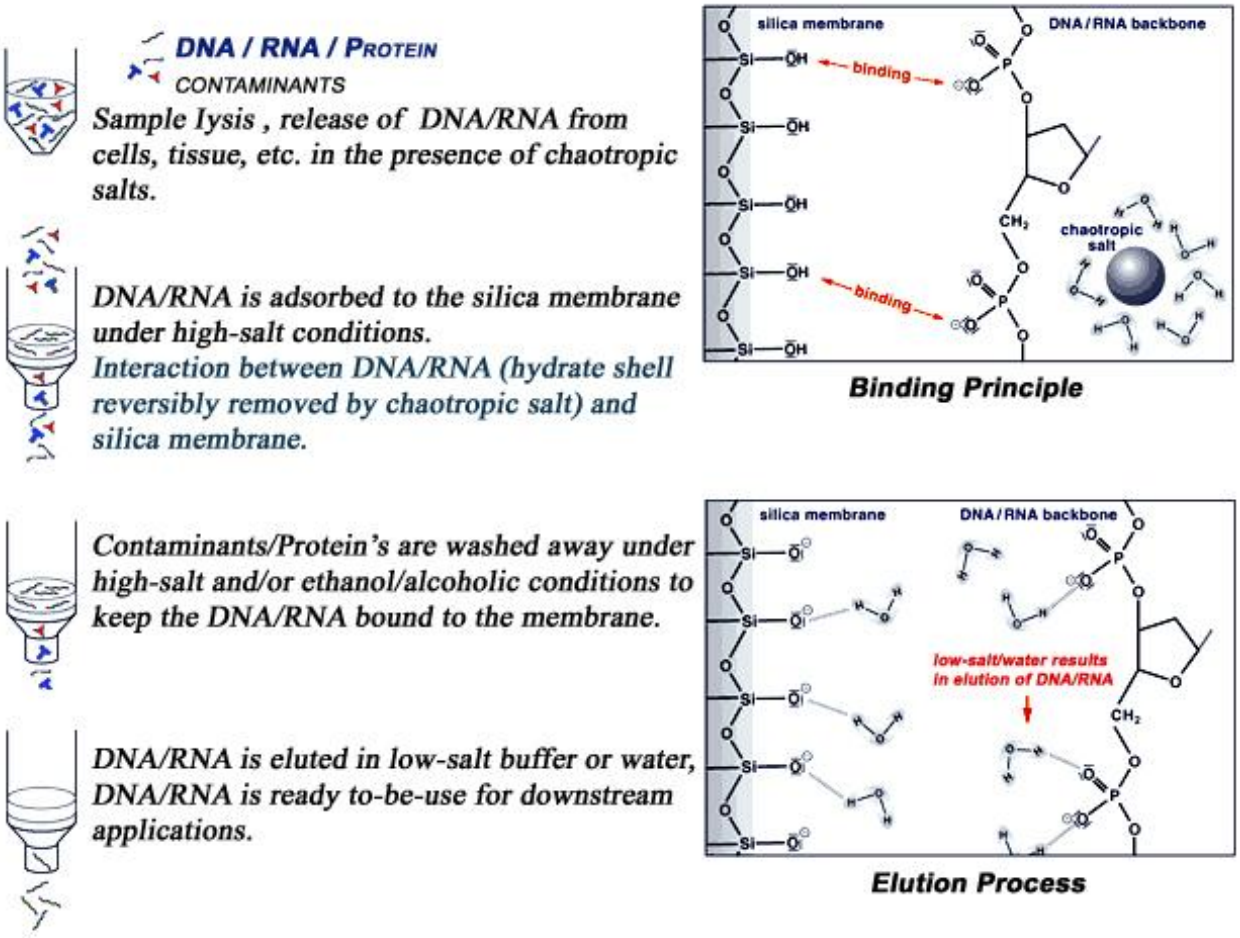
Geno Sen's® Tissue RNA Extraction Mini Kits provide's the fastest and easiest way to purify Tissue RNA for reliable use in amplification/qPCR technologies. Tissue RNA can be purified from **Soft or Hard Tissue's like Liver ,Spleen, Thymus, Heart, Kidney, Brain** etc. Samples may be fresh or frozen.

Geno Sen's® Tisuue RNA Extraction Mini Kits are general purpose kits which can be used for isolation of Tissue RNA from a wide variety of Tissue Specimens but performance cannot be guaranteed for different specimens.

Geno Sen's® Tissue RNA extraction Mini Kits represent's a well established general-purpose technology for RNA extraction. The kit combines the selective binding properties of a silica gel-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 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 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® Tissue RNA Extraction Mini Kit.

Basic Principle of RNA/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 RNA before loading the sample onto the spin column. RNA 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.

Removal of residual contaminants

RNA, 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 RNA. Optimized wash conditions ensure complete removal of any residual contaminants without affecting RNA binding.

Elution with Buffer MEL

Buffer MEL is prepared in RNAse & 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 RNA, elution with RNAse -free water instead of Buffer MEL is recommended.

Co-purification of RNA with DNA

The Geno Sen's® Tissue RNA Extraction Mini Kit is not designed to separate RNA from the DNA, and both will be purified in parallel if present in the sample. 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 RNA extraction & is not optimized for DNA extraction as the yields for DNA will be low. For DNA extraction there is a separate optimized Kit Available. Cat. No. 98024 & 98025.

Sample volumes

Geno Sen's® spin columns can bind RNA upto 50kb in length. Actual yield will depend on sample size, sample storage, and RNA titer. The procedure is optimized for use with maximum 50 mg of tissue samples.

Lysis

Carrier RNA, Tissue Lysis Buffer & The lysis Buffer MBTR are provided in the kit for the Lysis of the sample. The Tissue is first Grinded & then lysed with the Tissue lysis Buffer under the highly denaturing conditions provided by The Tissue Lysis Buffer & then subjected to further lysis with Buffer MBTR to ensure isolation of intact RNA. If very low copy RNA is expected then proteinase K (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 Tissue Lysis Buffer

Addition of internal controls

Using the Geno Sen's® Tissue 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 can be added to the lysis buffer MBTR. 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 RNA isolated from Tissue samples normally depend upon the sample used for extraction. A normal person's 50 mg will typically provide a yield of 3-12 µg of RNA with the Geno Sen's® Tissue RNA Extraction Mini kit. Elution with 50µl of Buffer MEL is recommended as it will ensure elution of at least 90% RNA bound to the membrane.

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® Tissue RNA Extraction Mini protocols should be performed quickly and at room temperature.

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

The Geno Sen's® Tissue 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 MBTR*

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 MBTR for precipitate, and if necessary incubate at 80°C until the precipitate is dissolved. Calculate the volume of Buffer MBTR–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 MBTR*: **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.

Geno Sen's® Viral RNA Extraction Mini manual 01/2012

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

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

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

No. Samples	Vol. Buffer MBTR (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 MBTR. (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 MBTR & 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 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
98004	50	20 ml	16 ml	36 ml
98005	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
98004	50	12.5 ml	50 ml	62.5 ml
98005	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.

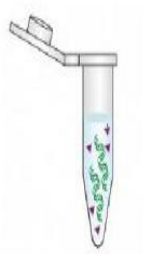
Spin protocol

■ 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

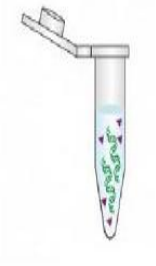
Geno Sen's® Mini columns will fit into most standard 1.5 ml or 2 ml microcentrifuge tubes.
Adequate Collection tubes has been provided for all the steps. However if required additional 2 ml collection tubes are available separately against orders.
Centrifugation at full speed will not affect RNA yield.
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.

Protocol at a glance.



Lysis Of the Tissue Sample

a) Grind the tissue in PBS or use directly & Centrifuge the same at 6000 r.p.m.
b) Discard the supernatant & add 200 µl of TLD.
c) Incubate at 65°C for 45-60 minutes.
d) Vortex for 30 secs.
e) Keep checking for clearance of the solution & Vortexing in between if required.
f) Solution should get cleared out before you start the next step or else incubate for some more time.



Further Lysis of the Sample

a) **Add 6 µl of reconstituted Carrier RNA (polyA) to the Lysate and vortex briefly**
b) **Add 600 µl of Buffer MBTR. Pulse Vortex for 15 secs.**
c) **Incubation at 65°C for 05 minutes.**
d) **Add 600 µl of ethanol & mix by pulse vortexing.**

Binding of the Nucleic Acids to the Silica Membrane

a) **Load The above Complete Lysate to the column.**
b) **Centrifuge at 12000 r.p.m. for 2 minute.**
c) **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

a) **Add 500 µl of MWB W2.**
b) **Centrifuge at 12000 r.p.m. for 2 minute.**
c) **Centrifuge again at 13000 r.p.m. for 3 minute.**
d) **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® Tissue RNA Extraction Mini Kit.

Detailed Spin Protocol

This protocol is for purification of Tissue RNA upto 50 mg of Soft Tissue using a microcentrifuge.

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 11.
- Heat **THE BUFFER MBTR** in a water Bath to 60°C-80°C before addition of Carrier RNA. This will increase the yield.
- Add carrier RNA reconstituted in Buffer MEL to Buffer MBTR according to instructions on page 10-11.

Procedure for Extraction of Tissue RNA

1. Grind 10-50 mg of Soft Tissue in 1x PBS or using a Bead Beater Grind the same.

2. Centrifuge at 10000 r.p.m. for 2 Minutes.

3. Discard the supernatant & Wash with 500µl of 1X PBS.

4. Centrifuge at 10000 r.p.m. for 2 Minutes.

5. Discard the supernatant.

6. Add 200 µl of Tissue Lysis Buffer TLD to the above microcentrifuge tube.

7. Vortex Vigrously for about 30 secs-2 minutes so that the solution becomes homogenized.

8. Incubate at 60°C for 45-60 min in a pre-heated Heating Block or water bath.

Keep checking for clearance of the solution & Vortexing in between. Solution should get cleared out before you start the next step or else incubate for some more time.

9. Pipet 6 µl of prepared Carrier RNA (polyA) to the above microcentrifuge tube.

Carrier RNA (polyA) should never be mixed with Buffer TLD & should always be dispensed separately.

10. Add 600 µl of Buffer MBTR 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 MBTR to yield a homogeneous solution.

11. Incubate at 60°C for 5 min in a pre-heated Heating Block or water bath.

RNA lysis is complete after 5 min at **60°C**. Longer incubation times have no effect on the yield or quality of the purified RNA.

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

13. Add 600 µl of ethanol to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid.

14. **Carefully apply the entire Lysate from step 13 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 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.

15. 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 50mg.

16. 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.**

17. 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 17, and then continue with step 18.

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.

18. Place the Spin column in a clean (RNaes & RNAes 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 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%.

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

When larger yields of RNA 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 RNA 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 RNA in the elute		
a)	Carrier RNA not added to Buffer MBTR	Reconstitute carrier RNA in Buffer MEL and mix with Buffer MBTR 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 MBTR–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)	Inefficient protein denaturation in Buffer MBTR	Precipitate, formed in Buffer MBTR–carrier RNA after mixing. Redissolve the precipitate by heating at 80°C. and repeat the procedure with a new sample.
e)	Buffer MBTR prepared incorrectly	Check Buffer MBTR for precipitate. Dissolve precipitate by incubation at 80°C.
f)	No ethanol added to the lysate (step 5)	Repeat the purification procedure with a new sample.
g)	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.
h)	Isoproponal used at Step 13 Instead of Ethanol.	Repeat the purification procedure with a new sample. Use only Ethanol at Step 13.
i)	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.
j)	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.
k)	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.
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. Also ensure that 70% ethanol is not used.
m)	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.
n)	RNAse contamination in Buffer MEL	Discard contaminated Buffer MEL. Repeat the purification procedure with a new sample and a fresh tube of Buffer MEL.

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)	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 Enzymes used i.e. Taq RNA polymerase etc.	If enzymes are changed, it may be necessary to first check the activity of enzymes with some known samples.
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 RNA.

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	The Tissue lysis was not carried out properly or excess amount of tissue was used than the recommended. Repeat after correcting the above issues.
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 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 RNAse free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNAse-free water (see "Solutions", page 37). Alternatively, chloroform resistant 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. 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 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 RNA: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® Tissue RNA extraction kit 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

If you have any questions or experience any difficulties regarding the Geno Sen's® Tissue 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
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® Tissue RNA Extraction Mini Kits is tested against predetermined specifications to ensure consistent product quality.

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

Ordering Information

RNA EXTRACTION KITS			
S.No.	**Cat. No.**	**Preps**	**Product**
1.	**98021**	**50**	**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.**
2.	**98022**	**250**	**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.**
3.	**98024**	**50**	**Geno Sen's® Tissue DNA Extraction Mini Kit for extraction of DNA from Soft or Hard Tissue i.e. Liver ,Spleen, Thymus, Heart, Kidney, Brain** etc.
4.	**98025**	**250**	**Geno Sen's® Tissue DNA Extraction Mini Kit for extraction of DNA from Soft or Hard Tissue i.e. Liver ,Spleen, Thymus, Heart, Kidney, Brain** etc.

RNA EXTRACTION KITS			
S.No.	**Cat. No.**	**Preps**	**Product**
1.	**98001**	**50**	**Geno Sen's® Viral RNA extraction Mini Kit for Viral RNA purification from serum, plasma, and body fluids, cell culture supernatants, C.S.F.**
2.	**98002**	**250**	**Geno Sen's® Viral RNA Extraction Mini Kit for Viral RNA purification from serum, plasma, and body fluids, cell culture supernatants, C.S.F.**
3.	**98004**	**50**	**Geno Sen's® Tissue RNA Extraction Mini Kit for extraction of RNA from Soft or Hard Tissue i.e. Liver ,Spleen, Thymus, Heart, Kidney, Brain** etc.
4.	**98005**	**250**	**Geno Sen's® Tissue RNA Extraction Mini Kit for extraction of RNA from Soft or Hard Tissue i.e. Liver ,Spleen, Thymus, Heart, Kidney, Brain** etc.
5.	**98027**	**50**	**Geno Sen's® Total RNA Extraction Mini Kit for extraction of RNA from Whole Blood**
6.	**98028**	**250**	**Geno Sen's® Total RNA Extraction Mini Kit for extraction of RNA from Whole Blood**

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 RNA.
2.	99002	500 ml.	Geno Sen's® SputoLyse Solution for de-contamination of sputum samples before extracting RNA.
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®RNAse
7.	99012		Geno Sen's®RNAse
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.