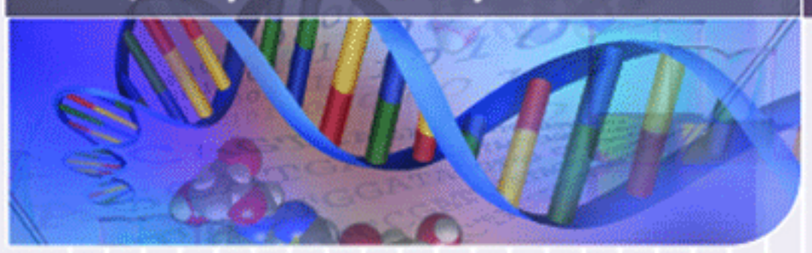


Genetic Tools & Reagents

Product Manual

Omni-Pure™ Viral RNA Purification System

Catalog No:40-3650-01 100 Purifications Kit

Catalog No:40-3650-05 500 Purifications Kit

Gene Link™

Omni-Pure™ Viral RNA Purification System

Contents

Materials Supplied

Omni-Pure™ Viral RNA Purification Systems

Omni-Pure™ Viral RNA Purification System			
	Product	**Catalog No.**	**Size***
❑	Omni-Pure™ Viral RNA Purification System	40-3650-01	100
❑	Omni-Pure™ Viral RNA Purification System	40-3650-05	500
*Unit of size is purification performed			

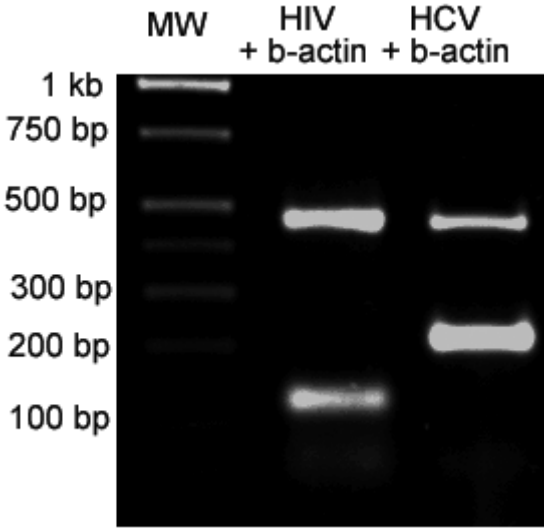
Omni-Pure™ Viral RNA Purification System				
Product	**Catalog No.**	**Size**	**Catalog No.**	**Size**
	❑		❑	
Omni-Pure™ Viral RNA Purification System	40-3650-01	100	40-3650-05	500
Materials Supplied				
VR1 Solution; Cell Lysis Solution	40-3651-04	40 ml	40-3651-20	200 ml
VR2 Solution; RNA Wash Solution 4 X concentrate supplied. Reconstitution Required*	40-3652-01	10 ml*	40-3652-05	50 ml*
VR3 Solution; RNA Elution Solution	40-3653-01	10 ml	40-3653-03	30 ml
Spin Columns	40-4121-01	100	40-4121-01	5X 100

*VR2-Solution 4 X Concentrate; Reconstitution Required Prior To Use			
Reconstitution Procedure			
Product	**Catalog No.**	**Size**	**Volume of 100% Ethanol to Add**
VR2 Solution; RNA Wash Solution 4 X concentrate supplied	40-3652-01	10 ml	30 ml
VR2 Solution; RNA Wash Solution 4 X concentrate supplied	40-3652-05	50 ml	150 ml

Omni-Pure™ Viral RNA Purification Systems

Gene Link provides a rapid purification system for extraction of viral RNA from human bodily fluids including blood. Viral RNA is captured on a special membrane and then eluted in a low volume for direct use in qualitative and quantitative amplification protocols for detection of a pathogen. The Viral RNA purification system is ideal for small volumes of human bodily fluid samples, i.e., serum, plasma and CSF. Using the easy spin column format, purification of HIV, HCV and other RNA viruses is easily accomplished in less than 30 minutes and ready for RT-PCR amplification.

- ◇ No hazardous or toxic reagents
- ◇ Quick 30 minute protocol
- ◇ Suitable for all molecular biology applications
- ◇ Easy spin column format



Viral RNA purification followed by amplification for HIV and HCV specific fragments. The top fragment of ~500 bp is an internal control from human genomic DNA.

Product Description

Introduction

The Omni-Pure™ Viral RNA Purification System provides an easy-to-use kit of optimized reagents and a rapid protocol to yield purified Viral RNA. The purified RNA is suitable for all molecular biology applications and has been thoroughly tested. The Omni-Pure™ Viral RNA purification system uses non-hazardous reagents and especially does not use the classic phenol-chloroform protocol or any chaotropic salts.

This Viral RNA Purification System is not designed to separate RNA from DNA. DNA in samples will be co-extracted to some extent. Smaller than 200 bp RNA are not extracted quantitatively.

Sample Type

The Omni-Pure™ Viral RNA Purification System is specifically designed for cell free sample types. Any sample that contains cells should first be centrifuged to pellet the cells and the supernatant used for viral RNA purification. Appropriate sample types are serum, plasma, and other cell free samples.

This kit is particularly formulated to extract and purify RNA from 200 µl sample volumes and smaller sample sizes with almost all manipulations being carried out in 1.5 ml tubes. Multiple samples can be processed at the same time. Viral RNA is obtained in less than 30 minutes.

Proper sample handling and storage protocols should be established to prevent RNA degradation. It is essential that completely RNase free working space and equipment be used.

Decontamination

All human and animal samples used for purification of RNA and DNA should be considered infectious. Proper decontamination protocols should be followed for eventual disposal. All waste materials should be properly decontaminated and disposed following institutional guidelines. A standard decontamination protocol is given in this manual for information only and is not a substitute for any other protocol established by the institution or OSHA. Household bleach is a readily available and effective disinfectant. Extended heating at 80^{o}C to 100^{o}C for 20 minutes or longer denatures and inactivates most pathogens.

Genotyping Method & Sample Requirements

PCR based genotyping requires low quantities of RNA or DNA. Usually less than a few hundred microliter of sample is required.

Omni-Pure™ Viral RNA Purification System

Quick Protocol: Purification of Viral RNA*

from plasma, serum, urine, cell-free bodily fluids & cell culture supernatants

Catalog No: 40-3650-XX

Sample volume example: 200 µl (scale up or down as required)

Please consult manual for details and background information. Sterile RNase free reagents, disposable pipet tips and tubes, and working environment required to obtain good RNA yield.

A. Sample & Reagent Preparation

1. Component VR2 of this kit is supplied as a 4X concentrate. Add 3 volumes of 100% ethanol prior to first use.
2. Centrifuge bodily fluid samples at 12K rpm for 20 seconds to pellet cells. This kit is specially formulated to process cell free samples. Use supernatant, serum or plasma as sample.
3. Label two set of appropriate number of sterile RNase free 1.5 ml tubes. To one set add 400 µl of VR1 buffer to each tube. Other empty set to be used for RNA elution.
4. Assemble appropriate number of spin column with collection tubes. Label appropriately.

B. Viral RNA Purification

1. Using a sterile RNase free filter tip pipet transfer 200 µl of sample to tubes containing 400 µl of VR1 buffer (Prepared in step A3 above). Mix thoroughly by gentle vortexing.
2. Incubate at room temperature for 5 minutes.
3. To each tube add 600 µl of 100% ethanol. Mix thoroughly by gentle vortexing and transfer contents immediately to spin column with collection tubes. (Prepared in step A4 above).
4. Centrifuge at 12K rpm for 5 minutes. Empty the collection tube by discarding the filtrate.
5. Add 400 µl of diluted VR2 (see A1 above) to the spin column.
6. Centrifuge at 12K rpm for 5 minutes. Discard the filtrate. **The spin column should not have any VR2 buffer as left over. Spin again if there is any trace of liquid. Spin column should be almost dry.**
7. Replace collection tube below the spin column with an appropriately labeled sterile RNase free 1.5 ml tube. (Prepared in step A3 above).

C. RNA Elution

1. Using a sterile RNase free filter tip pipet add 50 µl of VR3 Elution solution directly to the filter of the spin column. Let stand at room temperature for 5 minutes.
2. Centrifuge at 12K rpm for 2 minutes to collect purified RNA in the collection tube.
3. Purified RNA should be amplified immediately, or stored at –20 °C or preferably at –70 °C.

⚠ Treat all bodily fluids, including blood and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in the manual.

⚠ Always use filter barrier pipette tips to prevent cross contamination.

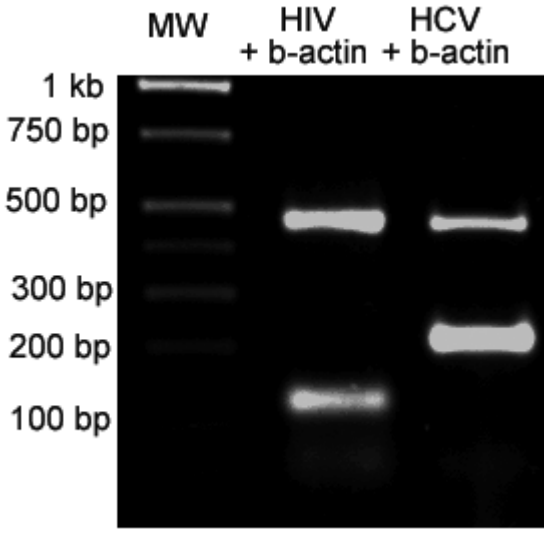
- Prepare appropriately labeled tubes prior to starting procedure.
- It is convenient to add samples to tubes containing pre-aliquoted reagents.

- All samples should be at room temperature before processing.
- All centrifugation is carried out at room temperature.
- Purified RNA should be amplified immediately, or stored at –20 °C or preferably at –70 °C.

***This Viral RNA Purification System is not designed to separate RNA from DNA. DNA in samples will be co-extracted to some extent. Smaller than 200 bp RNA are not extracted quantitatively.**

Sample Results and Interpretation

Viral RNA purification is usually followed by RT-PCR amplification to check the presence or absence of the particular viral RNA in the sample. There are RT-PCR kits available to perform the amplification. It is advisable to include Beta actin is included in the reaction as an internal control to verify faithful amplification protocol. The beta actin amplification fragment is of ~500bp and should be seen in all amplification reactions. The lower fragment of ~100 bp for HIV and 200bp represents specific amplification from *Hepatitis C Viral* RNA. *patitis C Viral* RNA template.



Viral RNA purification followed by amplification for HIV and HCV specific fragments. The top fragment of ~500 bp is an internal control from human genomic DNA.

Troubleshooting

Problem	Protocol Step	Reasons and Suggestions
Low RNA yield	Sample preparation	RNA in sample was degraded prior to RNA purification. 1. Sample was not adequately protected from RNA degradation. RNase free environment, disposable plastic wares, equipment and reagents are an absolute requirement for obtaining good RNA yield. 2. Ensure that sample was transported rapidly and collected in RNase free container. 3. Ensure that sample was stored properly; preferably frozen if the sample is not processed within 24 hrs.
Low RNA yield	Cell lysis	RNA in sample was not lysed completely. 1. Ensure that correct amount of solution VR1 was added. 2. The sample was not cell free. Presence of too many cells release DNA and proteins that compete with RNA extraction.
Low RNA yield	RNA Elution	RNA in sample was not completely recovered. 1. The spin column should be almost dry before elution of RNA. Ensure that there is no left over reagent from the previous step. If required perform an additional spin. 2. The RNA was completely eluted. Perform one more elution and pool with the previous elution.
Low or no RNA yield		RNase contamination. 1. The single most common reason for problems with RNA purification is not adequately performing the purification in an RNase free manner. Follow the protocol given in the appendix for 'Avoiding RNase Contamination."

Appendix

Protocols for Avoiding Ribonuclease Contamination

Ribonuclease present in the environment and within your sample can rapidly degrade RNA resulting in low yield and poor quality. To avoid RNA degradation we suggest the following:

1. Have all equipment, reagents and disposable tips and tubes arranged and labeled prior to start working. We strongly encourage that all tubes are pre-labeled and if possible pre-aliquot reagents.
2. All materials coming in contact with the sample must be sterile and RNase-free. Use sterile disposable pipets, pipet tips and sterile disposable tubes whenever possible. Use sterile technique at all times. Clean all equipment and with 70% ethanol prepared with DEPC treated RNase-free deionized water.
3. Wear gloves during the entire procedure to avoid introducing RNase contaminants into the sample from your hands.
4. The 70% ethanol solution used for RNA isolation should be made with diethyl pyrocarbonate (DEPC) treated water. DEPC water is made by adding DEPC to a final concentration of 0.1%. Observe proper safety precautions (i.e. use a fume hood) when using DEPC, which is a powerful acylating agent and forms ethyl carbamate, a potent carcinogen, when exposed to ammonia. Note: DEPC in aqueous solutions hydrolyzes over time, even if refrigerated, or upon autoclaving or heating to 70° C for 1 h.
5. Reserve bench area, reagents, a set of pipets and possibly a centrifuge exclusively for RNA work. As discussed below, process tissue samples with Lysis Buffer or freeze tissue immediately after harvesting to avoid mRNA degradation.

Decontamination of Bodily Fluids and Tissue Samples

All human and animal samples used for purification of DNA & RNA should be considered infectious and proper decontamination protocol should be followed for eventual disposal. The following protocol is an easy and tested decontamination protocol.

Bodily Fluids

1. Prepare 1 L of 1x bleach solution in a large narrow mouth bottle. Keep the bottle capped. See recipe.
2. Transfer all liquid waste to this bottle. You can add up to 300 ml waste to this 1 L bleach solution.
3. At the end of the DNA purification protocol and after at least 1 hour decontamination, this bleach solution can be safely discarded in a regular sink/sewer. Precipitates appear after longer storage.
4. Let cold water run for 3-5 minutes to completely rinse, dilute and wash the sink.

Solid Waste

1. All solid wastes should be disposed of in orange biohazard bags for eventual autoclaving and disposal.
2. All sharps should be disposed in sharps container and disposed of after autoclaving.
3. Paper towels, pipet tips and disposable plasticware should be treated as solid waste.

- All bodily fluids and tissue samples are to be considered infectious and hazardous.
- Wear gloves and protective clothing to prevent any exposure.
- All waste materials should be properly decontaminated and disposed following institutional guidelines.
- The decontamination protocol given here is for information only and is not a substitute for any other protocol established by your institution or OSHA.

- Household bleach is a readily available and effective disinfectant.
- Common household bleach contains 5% sodium hypochlorite. This is a convenient 10X solution.
- Extended heating at 80°C to 100°C for 20 minutes or longer denatures and inactivates most pathogens.

Recipe

1x Bleach Solution	
Dilution of household bleach	
10x Bleach	Water
100 ml	900 ml

Size and MW of Various Nucleic Acids

Nucleic acid	Length in bases or base pairs	MW, Daltons
RNA		
tRNA (E.coli)	75	2.5×10^4
5S rRNA	120	3.6×10^4
16S rRNA	1700	5.5×10^5
18S rRNA	1900	6.1×10^5
23S rRNA	3700	1.2×10^6
28S rRNA	4800	1.6×10^6
DNA		
pBR322 DNA	4361	2.8×10^6
SV40	5243	3.5×10^6
PhiX174	5386	3.6×10^6
Adenovirus 2 (Ad2)	35937	2.8×10^7
Lambda phage	48502	3.1×10^7
Escherichia coli	4.7×10^6	3.1×10^9
Saccharomyces cerevisiae	1.5×10^7	9.9×10^{10}
Dictyostelium discoideum	5.4×10^7	3.6×10^{10}
Arabidopsis thaliana	7.0×10^7	4.6×10^{10}
Caenorhabditis elegans	8.0×10^7	5.3×10^{10}
Drosophila melanogaster	1.4×10^8	9.2×10^{10}
Gallus domesticus (chicken)	1.2×10^9	7.9×10^{11}
Mus musculus (mouse)	2.7×10^9	1.8×10^{12}
Rattus norvegicus (rat)	3.0×10^9	2.0×10^{12}
Xenopus laevis	3.1×10^9	2.0×10^{12}
Homo sapiens	3.3×10^9	2.2×10^{12}
Zea mays	3.9×10^9	2.6×10^{12}
Nicotiana tabacum	4.8×10^9	3.2×10^{12}

Reference

1. *Ausubel, F.M., et al., Current Protocols in Molecular Biology, John Wiley and Sons, New York, 1988.*

Spectrophotometric Determination of RNA & DNA Concentration

Measuring the optical density (OD) or absorbance at 260 nm (A_{260}) in a UV spectrophotometer is a relatively accurate method for calculating the concentration of DNA in an aqueous solution if a standard curve is meticulously prepared. An A_{260} of 1.0, using a 1 cm path length, corresponds to a DNA concentration of 50 µg/ml for double stranded DNA, 40 µg/ml for single stranded DNA and RNA, and 33 µg/ml for oligonucleotides. However, this method is not suitable for determining concentrations of dilute solutions of DNA, as the sensitivity of this method is not very high. For reliable readings, the concentration of double stranded DNA must be greater than 1 µg/ml.

A simple, inexpensive method for the estimation of nanogram quantities of DNA is described in the following section. We recommend the use of agarose gel electrophoresis for routine approximate determination of DNA concentration.

Ordering Information

Omni-Pure™ Genomic DNA Purification Systems		
Product	**Catalog No.**	**Size***
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Universal DNA Purification System	40-4070-01	100
Omni-Pure™ Universal DNA Purification System	40-4070-05	500
Omni-Pure™ Universal DNA Purification System	40-4070-10	1000
*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.		

Related Products Ordering Information

Omni-Pure™ DNA & RNA Purification Systems		
Product	**Catalog No.**	**Size***
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500
*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.		

Omni-Clean™ Gel DNA Purification and Concentration Systems		
Product	**Catalog No.**	**Size***
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500
*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.		

Visit www.genelink.com for pricing and ordering information.