Genetic Tools & Reagents

Product Manual

Omni-Pure™ Microbial DNA Purification System

Catalog No:40-3700-01 100 Purifications Kit

Catalog No:40-3700-05 500 Purifications Kit

Gene Link™

Omni-Pure™ Microbial DNA Purification System

Gene Link™

Contents

Materials Supplied

Omni-Pure™ Microbial DNA Purification System

Omni-Pure™ Microbial DNA Purification System			
	Product	**Catalog No.**	**Size***
❑	Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
❑	Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
*Unit of size is purification performed			

Omni-Pure™ Microbial DNA Purification System				
Product	**Catalog No.**	**Size**	**Catalog No.**	**Size**
	❑		❑	
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100	40-3700-05	500
Materials Supplied				
MD1 Solution; Microbial Prep Solution	40-3703-03	30 ml	40-3703-15	150 ml
MD2 Solution; Cell Lysis Solution	40-3704-04	40 ml	40-3704-20	200 ml
VD2 Solution; DNA Wash Solution 4 X concentrate supplied. Reconstitution Required*	40-3725-25	25 ml*	40-3725-12	120 ml*
VD3 Solution; DNA Elution Solution	40-3726-01	10 ml	40-3726-04	40 ml
Spin Columns	40-4121-01	100	40-4121-01	5X 100

*VD2 Solution 4 X Concentrate; Reconstitution Required Prior To Use			
Reconstitution Procedure			
Product	**Catalog No.**	**Size**	**Volume of 100% Ethanol to Add**
VD2 Solution; DNA Wash Solution 4 X concentrate supplied	40-3725-25	25 ml	75 ml
VD2 Solution; DNA Wash Solution 4 X concentrate supplied	40-3725-12	120 ml	360 ml

Omni-Pure™ Microbial DNA Purification Systems

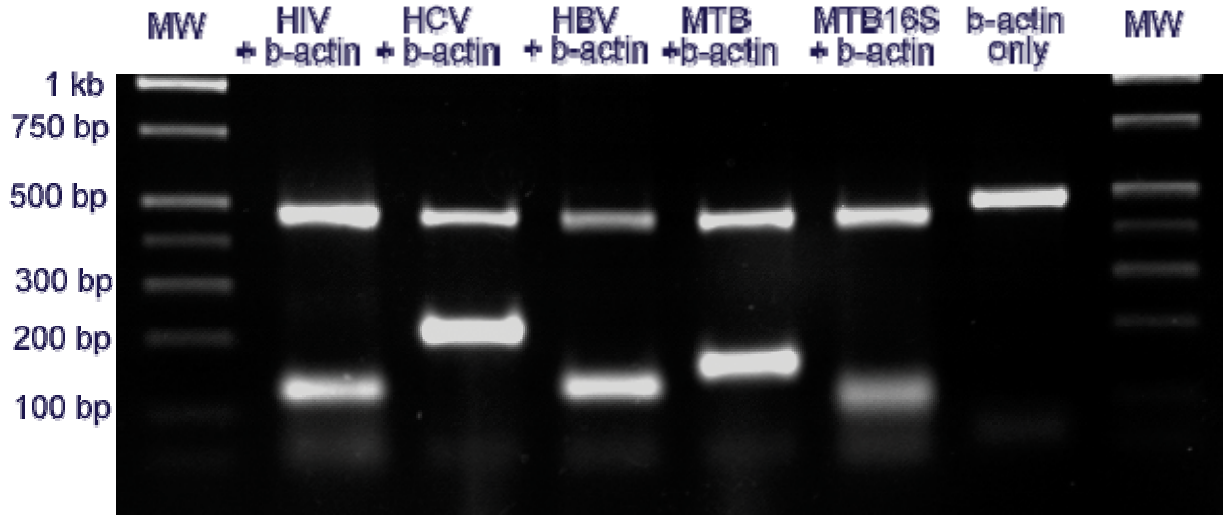
Gene Link provides a rapid purification system for extraction of Microbial DNA from human bodily fluids specifically sputum and BAL. Microbial DNA is captured on a special membrane and then eluted in a low volume for direct use in qualitative and quantitative amplification protocols for detection of a pathogen. The Microbial DNA purification system is ideal for small volumes of human bodily fluid samples, i.e., sputum, BAL and CSF. Using the easy spin column format, purification of DNA 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 and DNA and mycobacterium DNA purification using Omni-Pure™ purification systems followed by amplification of specific viral or mycobacterium and human genomic DNA fragments. The top fragment of ~500 bp is an internal control from human genomic DNA.

Product Description

Introduction

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

This Microbial DNA Purification System is not designed to completely eliminate and separate host genomic DNA from Microbial DNA. Host genomic DNA will be co-extracted to some extent. Smaller than 200 bp DNA are not extracted quantitatively.

Sample Type & Detection Methods

The Omni-Pure™ Microbial DNA Purification System is specifically designed for purification of *Mycobacterium tuberculosis* DNA. *Mycobacterium tuberculosis* is the causative agent of tuberculosis.

Classically the detection of MTB is performed by anti tuberculin test to detect immune response against mycobacterium tuberculosis, this is a surrogate marker used to determine a possible mycobacterial infection without knowing the organ(s) involved. Skin or tuberculin test: this is usually the first test that is performed to detect an immune reaction against the MTB pathogen.

Pulmonary TB is often detected by lung X-ray. Direct examination by smear microscopy of acid-fast bacilli in the sputum/bronchio-alveolar-lavage (BAL) of a patient indicates an active and contagious form of tuberculosis. Culture of the bacteria is still the gold standard using either conventional methods or Bactech from BD. In recent years, PCR technology has been used to identify the location of myco-bacterial infection using samples from Cerebro Spinal Fluid (CSF), tissue and lymph node biopsies, urine and even blood. PCR amplification of IS6110 insertion element DNA and RT-PCR of 16sRNA studies have been initiated to detect the presence of bacteria and the presence of live bacteria respectively in any sample.

One of the most efficient high-throughput procedures is that combines DNA or RNA extraction, amplification, and detection of pathogen DNA or RNA by real-time quantitative instruments. A real-time assay detects and quantitates the pathogen load. The detection limit of real time PCR is approximately 100 copies. A qualitative detection assay performed by running conventional gel electrophoresis can determine the presence or absence of the pathogen specific amplified fragment.

This kit is particularly formulated to extract and purify DNA from 500 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. Microbial DNA is obtained in less than 30 minutes.

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°C to 100°C for 20 minutes or longer denatures and inactivates most pathogens.

Omni-Pure™ Microbial DNA Purification System

Quick Protocol: Purification of Microbial DNA

from *Mycobacterium tuberculosis* (MTB). Sample of sputum/BAL and CSF.

All samples should be collected as per GLP protocols

Catalog No: 40-3700-XX

Sample volume example: 200 µl sputum or BAL (bronchio-alveolar-lavage)

All bodily fluid samples should be considered infectious and proper safety procedures should be followed.

A. Sample & Reagent Preparation

1. Component VD2 of this kit is supplied as a 4X concentrate. Add 3 volumes of 100% ethanol prior to first use.
2. Label two set of appropriate number of sterile RNase free 1.5 ml tubes. To each tube of this set add 200 µl of MD1 Microbial Prep Solution. To the second set add 300 µl of MD2 Cell Lysis solution. To be used for transfer of supernatant in step B3.
3. Assemble appropriate number of spin column with collection tubes. Label appropriately.

B. Microbial DNA Purification

1. Using a sterile filter tip pipet transfer 200 µl of sample to tubes containing 200 µl of MD1 Microbial Prep Solution (Prepared in step A2 above). Mix vigorously by vortexing for nearly 1 minute. Keep at room temperature for 20 minutes with occasional mixing.
2. Centrifuge at 12K rpm for 10 minutes.
3. Transfer supernatant using a pipet to appropriately labeled 1.5 ml tubes containing 300 µl MD2 Cell Lysis solution. (Prepared in step A2 above). Vortex gently.
4. Transfer contents to spin columns with collection tubes.
5. Centrifuge at 4K rpm for 5 minutes. Empty the collection tube by discarding the filtrate. Spin again if column is not dry.
6. Add 400 µl of diluted VD2 (see A1 above) to spin column.
7. Centrifuge at 12K rpm for 5 minutes. Discard the filtrate.
8. **Repeat steps 5 and 6 one more time. The spin column should not have any VD2 buffer as left over. Spin again if there is any trace of liquid. Spin column should be almost dry.**
9. Replace collection tube below the spin column with an appropriately labeled sterile 1.5 ml tube. (Prepared in step A3 above).

C. DNA Elution

1. Using a sterile RNase free filter tip pipet add 50 µl of VD3 DNA 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 DNA in the collection tube.
3. Purified DNA should be amplified immediately, or stored at –20 °C or preferably at –70 °C.

⚠ Sample decontamination and DNA extraction should be performed in a biological safety cabinet with unidirectional work flow for all procedures.

⚠ 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.

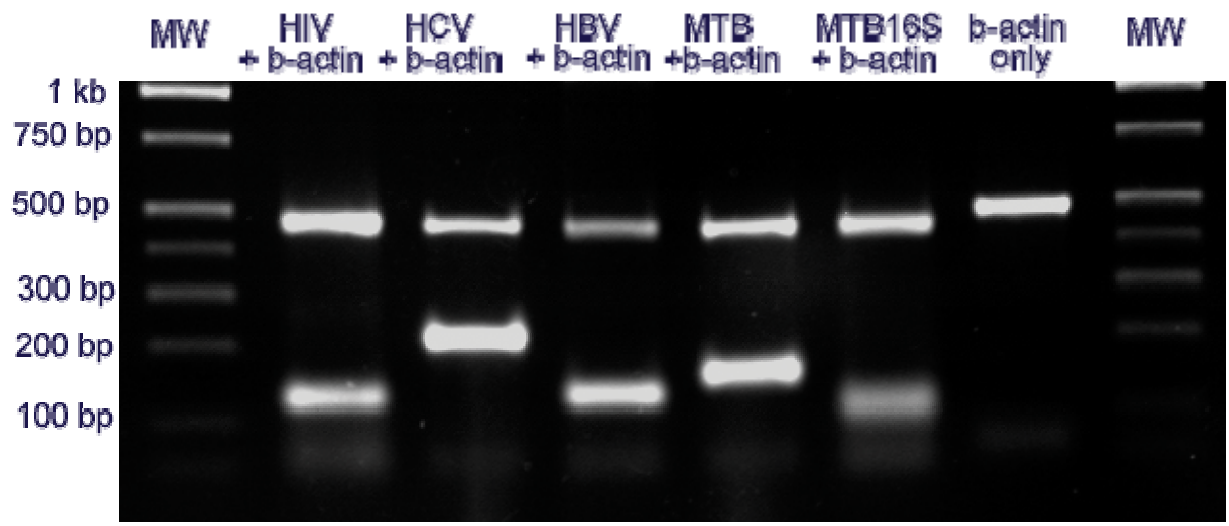
ⓘ
- All samples should be at room temperature before processing.
- All centrifugation is carried out at room temperature.
- Purified DNA should be amplified immediately, or stored at –20 °C or preferably at –70 °C.

ⓘ
- DNA yield varies and is dependant on titer. Usually 5 -10 µl of the purified DNA is sufficient to obtain amplification.

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

Sample Results and Interpretation

Microbial DNA purification is usually followed by amplification to check the presence or absence of the particular pathogen DNA in the sample. It is advisable to include beta actin in the reaction as an internal control to verify faithful amplification protocol.



Viral RNA and DNA and mycobacterium DNA purification using Omni-Pure™ purification systems followed by amplification of specific viral or mycobacterium and human genomic DNA fragments. The top fragment of ~500 bp is an internal control from human genomic DNA.

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.*

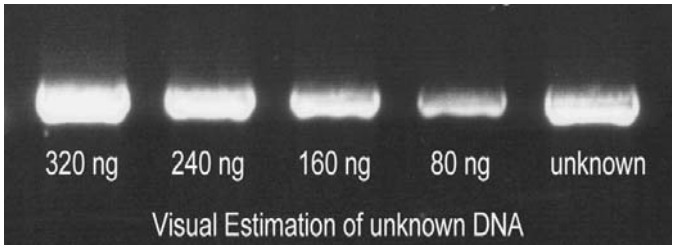
Spectrophotometric Determination of DNA Concentration

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.

Estimation of DNA Concentration by Agarose Gel Electrophoresis

The amount of DNA in a sample may be estimated by running the sample along side of standards containing known amounts of the same-sized DNA fragment. In the presence of ethidium bromide staining, the amount of sample DNA can be visually estimated by comparing the band intensity with that of the known standards.



An unknown amount of a 4 kb DNA fragment was run alongside known quantities (indicated in nanograms) of the same DNA fragment. As estimated by visual comparison with the known standards, the unknown sample contained 240-320 ng of DNA.

Ethidium bromide is a carcinogen. Follow Health and Safety Procedures established by your institution. Follow proper Hazardous Material Disposal procedures established by your institution.

- Use 0.1 µg of ethidium bromide solution for each ml of gel volume.

Ordering Information

Omni-Pure™ Viral & Microbial DNA & RNA Purification Systems		
Product	**Catalog No.**	**Size***
Omni-Pure™ Viral DNA Purification System	40-3720-05	50
Omni-Pure™ Viral DNA Purification System	40-3720-10	100
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-50	500
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-05	50
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-10	100
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-50	500
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-05	50
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-10	100
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-50	500
Omni-Pure™ Universal RNA Purification System	40-4091-05	50
Omni-Pure™ Universal RNA Purification System	40-4091-10	100
Omni-Pure™ Universal RNA Purification System	40-4091-50	500
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-05	500

**Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Related Products Ordering Information

Omni-Pure™ RNA Purification Systems		
Product	**Catalog No.**	**Size***
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-05	50
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-10	100
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-50	500
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-05	50
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-10	100
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-50	500
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-05	50
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-10	100
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-50	500
Omni-Pure™ Universal RNA Purification System	40-4091-05	50
Omni-Pure™ Universal RNA Purification System	40-4091-10	100
Omni-Pure™ Universal RNA Purification System	40-4091-50	500
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-05	500

**Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

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

**Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Related Products Ordering Information

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

* *Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

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