



Product Manual

Hepatitis B Virus Genemer™ Mix

**Primer Pair for amplification of HBV Specific DNA Fragment
Includes Internal Negative Control Primers and Template**

Catalog No.: 60-2007-12

Store at -20°C

For research use only. Not for use in diagnostic procedures for clinical purposes

Instruction Manual

Important Information

All Gene Link, Inc. products are for research use only. Not for use in diagnostic procedures for clinical purposes. Product to be used by experienced researchers appropriately trained in performing molecular biology techniques following established safety procedures. Additional qualification and certification is required for interpretation of results.

Material supplied

Hepatitis B Virus Genemer™ Mix

**Primer Pair for amplification of HBV Viral Specific Fragment
Includes Internal Negative Control Primers and Template**

Catalog No.: 60-2007-12

200 Reactions (2 nmols)

One tube containing the following lyophilized components

1. Specific Genemer™ pair.
2. Primer pair for negative internal control.
3. Template for negative control amplification.

Storage Instructions

1. Shipped lyophilized at room temperature.
2. Store at -20°C upon receipt.
3. Store at -20°C after reconstitution.

Product	Catalog Number	Size (reactions)
HBV Genemer™ Mix. Primer pair for specific amplification of a gene fragment. Includes internal negative control primer pair and template. Special optimized conditions may be required for certain amplifications.	60-2007-12	200 rxns

Introduction

Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world. The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). In addition, HBV carriers can transmit the disease for many years. More than 2,000 million people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus. Three quarters of the world's population lives in areas where there are high levels of infection.

Hepatitis B is caused by the hepatitis B virus (HBV), an enveloped virus containing a partially double-stranded, circular DNA genome, classified within the family hepadnavirus. The virus interferes with the functions of the liver while replicating in hepatocytes. The immune system is then activated to produce a specific reaction to combat and possibly eradicate the infectious agent. As a consequence of pathological damage, the liver becomes inflamed.

HBV may be the cause of up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among known human carcinogens. Hepatitis B virus is mainly transmitted via blood or blood products. In addition, semen, cervical secretions, saliva and tears also contain the virus, thus sexual and oral infections are also possible. In East Asia, perinatal infections are especially frequent. Infection is also possible via insufficiently sterilized instruments, tattooing and ear piercing.

Incubation Time: The incubation period is about 2-6 months. The incubation time is inversely related to the dose of virus received.

Detection Methods

Gene Link gene detection system uses the Polymerase Chain Reaction (PCR) to amplify the amount of a specific gene fragment DNA or cDNA derived from DNA present in a sample. Amplification of RNA virus templates is based on reverse transcriptase PCR (RT-PCR). The method is highly sensitive and is capable of detecting a few copies of template. Detection systems offered by Gene Link are for research use only and should not be used for clinical diagnosis.

Qualitative Detection System

This system detects the presence or absence of the specific target DNA or DNA template. It is highly sensitive and gives a positive or negative result. In someone who has never been exposed to the specific pathogen a negative result would occur. On occasion, a positive result is obtained in individuals who have overcome an infection and are clinically asymptomatic. This possibly indicates that minute quantities of pathogen are sometimes present.

Quantitative Detective System

Quantitative PCR (QPCR) also termed as Real Time PCR determines the quantity of pathogen template in the sample. This system requires the use of fluorescent probe and a real time fluorescent detection system. A specialized PCR system is employed that measures the quantity of amplification at each cycle of the process. The relative amplification per cycle is directly related to the initial amount of DNA or DNA template. This system provides an indication of the number of copies of the pathogen present. This result is used to determine what is known as "viral load" or the number of copies present.

Procedure

Genemer™ Mix Reconstitution

The Genemer™ Mix contains primer pair for specific amplification of a gene fragment. Includes internal negative control primer pair and template. Special optimized conditions may be required for certain amplifications.

Dissolve the supplied lyophilized Genemer™ Mix in 220 µl sterile TE. The primer concentration will be approximately 10 µM i.e. 10 pmols/µl.

The above reconstituted Genemer™ Mix contains internal negative control primer pair and template.

Amplification and Detection

This protocol assumes that amplification is being performed from purified DNA.

The HBV Genemer™ amplifies a 138 bp fragment.

Set up the following amplification files on a thermal cycler. Please refer to the instrument manufacturer's manual for setting up of the program.


HBV Amplification Profile		
Step	Temperature & Time	Cycles
Initial Denaturation	5 min at 94°C	1
Denaturation	15 seconds at 94°C	45
Annealing	30 seconds at 55°C	
Extension	30 seconds at 72°C	
Fill up	7 minutes at 72°C	1
Hold	Hold for infinity at 4°C	Hold

PCR*

Amplification of target fragment DNA requires optimization using varying amounts of the template based on its abundance. Generally less than 10 ng of template is sufficient for a successful amplification. It is a good strategy initially to amplify multiple amounts for optimization. Use known positive control DNA as one sample.


1. PCR Premix Preparation (PP). Label tube "PP"


PCR Premix Preparation (PP)		
Component	1 X 20 µl Rxn.	10 X 20 µl Rxns.
Sterile Water	9.5 µl	95 µl
10 X PCR Buffer	1.5 µl	15 µl
2.0 mM dNTP	2 µl	20 µl
Reconstituted Genemer™ Mix	1µl	10 µl
Taq Enzyme Mix (EM) See below for preparation	5 µl	50 µl
Template DNA (~100 ng)	1-2 µl	Add 1-2 µl DNA to each tube
Total Volume	20 µl	
Keep on ice during set up. After adding template start PCR File		

 Always use filter barrier pipette tips to prevent cross contamination.

●Recipe

TE Buffer pH 7.5 Composition
1 X TE Buffer pH 7.5
10 mM Tris-HCl pH 7.5
1 mM EDTA

 Program your thermal cycler instrument with an amplification profile prior to starting the amplification protocol. Consult your appropriate instrument manufacturer's manual.

 The PCR premix preparation protocol is written considering that more than one amplification reaction will be performed at the same time. If only one reaction is planned then there is no need to prepare the Taq Enzyme Mix (EM).

●Recipe

Gene Link PCR Buffer
1 X PCR Buffer
10 mM Tris-HCl pH 8.3
50 mM KCl
1.5 mM MgCl ₂
0.001% Gelatin

2. Taq Polymerase mix Preparation (EM). Label tube "EM"

Taq Enzyme Mix Preparation (EM)		
Component	1 X 20 µl Rxn.	10 X 20 µl Rxns.
Sterile Water	5 µl	50 µl
10 X PCR Buffer	0.5 µl	5 µl
Taq Polymerase	0.5 µl	5 µl
Add 5 µl to each reaction or to the premix as required		

●Recipe

1 X TAE Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA

Detection of amplification fragment by agarose gel electrophoresis.

Prepare a 1.5 % agarose gel containing 1 µg/ml ethidium bromide in TAE buffer.

1. Add 5 µl of 6X Orange G loading buffer to the amplified samples. Any other non-denaturing loading buffer can be substituted.
2. Load 10 µl of the amplified samples to the gel.
3. Electrophorese at 8 volts/cm for approximately 1 hour.
4. Visualize under UV. Obtain documentation by taking a gel picture.



Ethidium bromide is a carcinogen. Follow Health and Safety Procedures established by your institution.

Follow proper Hazardous Material Disposal procedures established by your institution.

●Recipe

6X Orange G loading buffer
10 mM Tris-HCl pH 7.6
60 mM EDTA
60% Glycerol
0.15% Orange G
0.03% Xylene Cyanol

Results and Interpretation

Beta actin is included in the reaction as an internal control to verify faithful amplification protocol. The beta actin amplification fragment is of ~500 bp and should be seen in all amplification reactions. The lower fragment of ~138 bp represents specific amplification from *Hepatitis B virus* DNA. It will be present in samples having *Hepatitis B virus* DNA template.

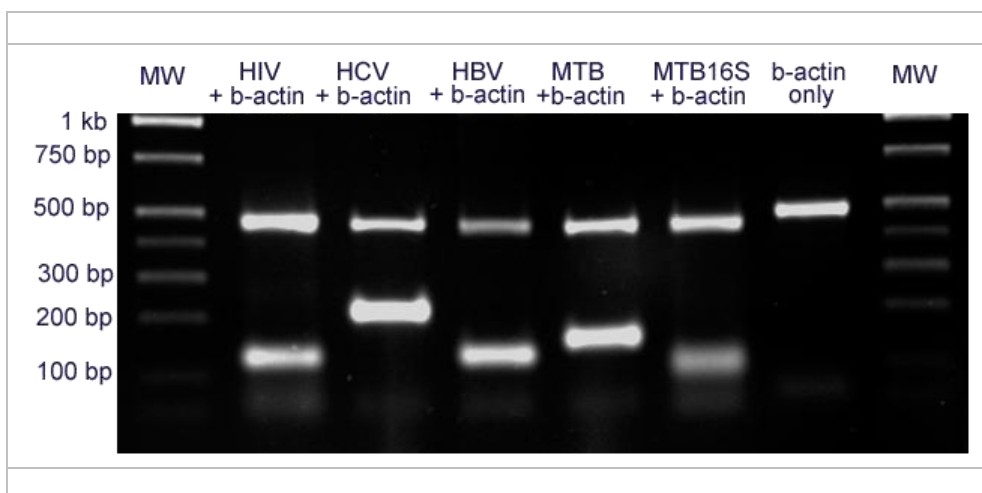
1. Two bands seen in the gel (500 bp and 138 bp):

The result is positive: report the sample as positive

2. Single band seen in the gel (500 bp):

The result is negative: report the sample as negative

Presence of *Hepatitis B virus* used to identify an HBV infection. These results also determine the efficacy of an ongoing antibiotic treatment, and help to identify potential drug resistant strains. However, as per the guidelines set forward by Center for Drug Control, viral presence, as determined by nucleic acid tests alone should not be used to determine the patient well being. Other markers of general patient health status should be used as well to ascertain the effects of the disease.



Troubleshooting

PCR/RT-PCR

Precautions:

1. Infectious agents should be handled as per cautious laboratory practices protocol
2. Purified nucleic acids should be amplified immediately, otherwise stored at -20°C for DNA and -70°C for RNA
3. Always use filter barrier pipette tips to prevent cross contamination
4. There should be a physical separation between the Pre-PCR and Post-PCR areas and the flow of movement of reagents and personal should always be unidirectional, i.e., always from Pre-PCR to Post-PCR and not otherwise. This will prevent the possibility of the laboratory being filled with amplicons as aerosol in air or equipment used, which can contaminate potential negative samples and give false positive results.
5. The items of the system should be stored as recommended.

Troubleshooting:

- No band in the positive control
 - a. Check the PCR conditions
 - b. Check the post-reconstitution storage of the reagents
 - c. Check the post purification storage of the nucleic acids
 - d. Check the sample collection protocol
 - e. Repeat the PCR reaction after checking the above
- Broad smear in place of sharp bands
 - a. Check the PCR conditions
 - b. Check the voltage in the agarose gel running apparatus
 - c. Repeat the PCR reaction after checking the above
- Two bands in the negative control
 - a. Cross contamination of samples/reagents
 - b. Clean the pre-PCR area with bleach followed by ethanol and water
 - c. Repeat the PCR reaction with fresh reagents

References

1. Yeh SH, Tsai CY, Kao JH, Liu CJ, Kuo TJ, Lin MW, Huang WL, Lu SF, Jih J, Chen DS, Chen PJ. Quantification and genotyping of hepatitis B virus in a single reaction by real-time PCR and melting curve analysis. *J Hepatol.* 2004 Oct;41(4):659-66.
2. Hanazaki K. Antiviral therapy for chronic hepatitis B: a review. *Curr Drug Targets Inflamm Allergy.* 2004 Mar; 3(1): 63-70. Review.
3. Zeuzem S. Overview of commercial HBV assays systems. *Methods Mol Med.* 2004; 95:3-13. Review.
4. Hu KQ. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat.* 2002 Jul;9(4):243-57.

Appendix

Pathogen DNA Extraction

1. Add 250 µl of DNA lysis buffer and 10 µl of Glass Beads. Vortex for 1 minute and incubate at RT for 5 minutes.
2. Transfer all the contents into an elution tube. Incubate at 65°C for 15 minutes.
3. Incubate at 95°C for 5 minutes.
4. Incubate at room temperature (RT) for 15 minutes.
5. Centrifuge at 5,000 rpm for 1 minute at RT.
6. Discard supernatant and add 500 µl of DNA wash buffer and vortex for 1 minute. Note: It is important to completely re-suspend the glass bead pellet at this step and in all subsequent steps.
7. Centrifuge at 5,000 rpm for 1 minute at RT. Discard supernatant and add 500 µl of DNA wash buffer and vortex for 1 minute.
8. Centrifuge at 5,000 rpm for 1 minute at RT.
9. Discard supernatant and add 35 µl of RNase free water and vortex for 1 minute.
10. Centrifuge at 5,000 rpm for 1 minute at RT to elute purified DNA.
11. Use eluted DNA for the PCR reaction.
12. The eluted DNA can be stored at this stage in -20°C.



Proper pathogen sample containment and decontamination protocols should be followed. DNA extraction should be performed in a biological safety cabinet with unidirectional workflow for all procedures.



The pathogen RNA purification protocol is specific for the Omni-Pure™ Viral RNA Purification System; Gene Link catalog No: 40-3650-XX.

Other reliable purification protocol and or product can be substituted.

Ordering Information

Genemer™: Primer pair for specific amplification of a gene fragment. Special optimized conditions may be required for certain amplifications.

Catalog No.	Product	Size*
60-2001-10	West Nile Virus (WNV) Genemer™	10 nmols
60-2002-10	Human Immunodeficiency Virus-1 (HIV-1) Genemer™	10 nmols
60-2003-10	Hepatitis C Virus (HCV) Genemer™	10 nmols
60-2004-10	Mycobacterium tuberculosis (MTB) Genemer™	10 nmols
60-2007-10	Hepatitis B Virus (HBV) Genemer™	10 nmols

*The quantity supplied is sufficient for 800 regular 20 µl PCR reactions

*Please visit www.genelink.com for other Genemer™ not listed here

Genemer™ Amplification Kit: Complete easy-to-use kit for reliable genotyping of a gene fragment. Includes a specific primer pair for gene or mutation specific amplification, optimized buffers and dNTPs and in most cases, control DNA.

Catalog No.	Product	Size
60-2001-11	West Nile Virus (WNV) Genemer™ Kit; 100 detections	1 Kit
60-2001-11S	West Nile Virus (WNV) Genemer™ Kit; 50 detections	1 Kit
60-2002-11	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Kit; 100 detections	1 Kit
60-2002-11S	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Kit; 50 detections	1 Kit
60-2003-11	Hepatitis C Virus (HCV) Genemer™ Kit; 100 detections	1 Kit
60-2003-11S	Hepatitis C Virus (HCV) Genemer™ Kit; 50 detections	1 Kit
60-2004-11	Mycobacterium tuberculosis (MTB) Genemer™ Kit; 100 detections	1 Kit
60-2004-11S	Mycobacterium tuberculosis (MTB) Genemer™ Kit; 50 detections	1 Kit
60-2007-11	Hepatitis B Virus (HBV) Genemer™ Kit; 100 detections	1 Kit
60-2007-11S	Hepatitis B Virus (HBV) Genemer™ Kit; 50 detections	1 Kit

*20 µl PCR reaction volume

*Please visit www.genelink.com for other Genemer™ Control DNA not listed here

Genemer™ Mix: Primer pair for specific amplification of a gene fragment. Includes internal negative control primer pair and template. Special optimized conditions may be required for certain amplifications.

Catalog No.	Product	Size*
60-2001-12	West Nile Virus (WNV) Genemer™ Mix	200 reactions
60-2002-12	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Mix	200 reactions
60-2003-12	Hepatitis C Virus (HCV) Genemer™ Mix	200 reactions
60-2004-12	Mycobacterium tuberculosis (MTB) Genemer™ Mix	200 reactions
60-2007-12	Hepatitis B Virus (HBV) Genemer™ Mix	200 reactions

*20 µl PCR reaction volume

*Please visit www.genelink.com for other Genemer™ not listed here

Genemer™ Control DNA: Cloned fragment of a particular gene for use with gene or mutation specific Genemer™ products. They are ideal genotyping templates for optimizing and performing control amplification with unknown DNA.

Catalog No.	Product	Size
60-2001-06	West Nile Virus (WNV) Genemer™ Control DNA	500 ng
60-2002-06	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Control DNA	500 ng
60-2003-06	Hepatitis C Virus (HCV) Genemer™ Control DNA	500 ng
60-2004-06	Mycobacterium tuberculosis (MTB) Genemer™ Control DNA	500 ng
60-2007-06	Hepatitis B Virus (HBV) Genemer™ Control DNA	500 ng

*Please visit www.genelink.com for other Genemer™ Kits not listed here

Omni-Clean™ and Omni-Pure™ Purification Systems: Facile and rapid purification of DNA and RNA from varied sources can be performed using the Omni-Pure™ series of DNA, RNA and plasmid purification systems.

Catalog No.	Product	Size
40-4010-01	Omni-Pure™ Genomic DNA Purification System	100
40-4010-05	Omni-Pure™ Genomic DNA Purification System	500
40-4010-10	Omni-Pure™ Genomic DNA Purification System	1000
40-3720-01	Omni-Pure™ Viral DNA Purification System	100
40-3720-05	Omni-Pure™ Viral DNA Purification System	500
40-3720-50	Omni-Pure™ Viral DNA Purification System	1000
40-3700-01	Omni-Pure™ Microbial DNA Purification System	100
40-3700-05	Omni-Pure™ Microbial DNA Purification System	500
40-3700-10	Omni-Pure™ Microbial DNA Purification System	1000
40-3650-01	Omni-Pure™ Viral RNA Purification System	100
40-3650-05	Omni-Pure™ Viral RNA Purification System	500
40-3650-10	Omni-Pure™ Viral RNA Purification System	1000
40-4110-10	Omni-Clean™ Gel DNA Beads Purification System	100
40-4110-50	Omni-Clean™ Gel DNA Beads Purification System	500
40-4120-10	Omni-Clean™ Gel DNA Spin Column Purification System	100
40-4120-50	Omni-Clean™ Gel DNA Spin Column Purification System	500
40-4130-10	Omni-Clean™ DNA Beads Concentration System	100
40-4130-50	Omni-Clean™ DNA Beads Concentration System	500
40-4140-10	Omni-Clean™ DNA Spin Column Concentration System	100
40-4140-50	Omni-Clean™ DNA Spin Column Concentration System	500

Genetic Tools and Reagents: Gene Link offers a wide variety of other molecular biology products, such as molecular weight markers, buffers, solutions and other genetic tools and reagents for all of your research needs.

Catalog No.	Product	Size
40-3005-01	Omni-Marker™ Universal unlabeled	100 µl
40-3005-05	Omni-Marker™ Universal unlabeled	500 µl
40-3005-10	Omni-Marker™ Universal unlabeled	1 ml
40-3006-01	Omni-Marker™ Low unlabeled	100 µl
40-3006-05	Omni-Marker™ Low unlabeled	500 µl
40-3006-10	Omni-Marker™ Low unlabeled	1 ml
40-3062-01	Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	100 µl
40-3062-05	Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	500 µl
40-3002-01	Loading Buffer 5X BPB/XC non-denaturing	100 µl
40-3002-10	Loading Buffer 5X BPB/XC non-denaturing	1 ml
40-3004-01	Loading Buffer 5X Orange G/XC non-denaturing	100 µl
40-3004-10	Loading Buffer 5X Orange G/XC non-denaturing	1 ml
40-5027-01	Loading Buffer 2X BPB/XC Denaturing for Sequencing	100 µl
40-5027-10	Loading Buffer 2X BPB/XC Denaturing for Sequencing	1 ml
40-5020-20	Hybwash A, Hybridization Wash Solution	200 ml
40-5021-10	Hybwash B, Hybridization Wash Solution	100 ml
40-5025-20	10x Washing buffer	200 ml
40-5026-10	10% Blocking solution	100 ml
40-5027-00	Seq. Loading buffer	1 ml
40-5031-10	10x AP Detection buffer	100 ml
40-5022-20	Lumisol™ I Hybridization Solution; contains formamide	200 ml
40-5023-20	Lumisol™ II Hybridization Solution; for non-toxic hybridizations	200 ml
40-5024-20	Lumisol™ III Hybridization Solution; for oligo probes	200 ml

Prices subject to change without notice

All Gene Link products are for research use only.