Product Manual

Hepatitis C Virus Genemer™ Mix

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

Catalog No.: 60-2003-12 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.

Gene Link™

Material supplied

Hepatitis C Virus Genemer™ Mix

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

Catalog No.: 60-2003-12 **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)
HCV 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.	60-2003-12	200 rxns

Introduction

Hepatitis C virus (HCV) is mainly transmitted via blood or blood products. However, sexual, oral and perinatal infections are also possible. Following a general malaise including appetite loss, vomiting and abdominal problems, about 10-20% of patients develop fever, exanthema (skin rash) as well as rheumatoid joint and muscle problems. Two to fourteen days later jaundice develops which may be accompanied by itching. Fulminant hepatitis occurs in about 1% of all infected patients and is frequently fatal. Some of hepatitis C patients develop chronic liver inflammation, which can progress to cirrhosis of liver or primary liver cell carcinoma.

Detection of HCV, involves serologic screening utilizing an enzyme immunoassay (EIA). Serologic assays for antibodies to HCV, i.e., anti-HCV antibodies, are 97% specific. However, these assays cannot be used to distinguish an acute infection from a chronic infection. In three successive versions of EIA, sensitivity has increased progressively. With older tests, some HCV infections may have been missed six to nine months after infection. The most recent third-generation EIA involves core protein and nonstructural proteins 3, 4, and 5; these can be used to detect antibodies within four to ten weeks after the onset of infection. False-negative results for the presence of HCV antibody can occur in persons with compromised immune systems, such as those with HIV-1 infection, patients with renal failure, and those with HCV-associated essential mixed cryoglobulinemia. False-positive EIA results can occur in persons without risk factors and in those without signs of liver disease, such as blood donors or health care workers. Recombinant immunoblot assay (RIBA-2) is used to confirm HCV infection. A positive immunoblot assay result is defined as the detection of antibodies against two or more antigens, and an indeterminate assay result is defined as the detection of antibodies against a single antigen. RIBA-2 is useful to confirm positive EIA results in low-risk populations.

Detection Methods

In recent rears HCV-RNA assays with PCR can be used to detect infection within one to three weeks of exposure. Compared with other tests, qualitative HCV-RNA tests based on the PCR technique have a lower limit of detection of fewer than 100 copies of HCV RNA per milliliter. HCV-RNA PCR tests are useful in confirming viremia, quantitating the vial load, assessing the treatment response, and examining patients with suspected false-negative results with antibody testing.

Gene Link gene detection systems use the Polymerase Chain Reaction (PCR) to amplify the amount of a specific gene fragment DNA or cDNA derived from RNA 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 RNA 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 a 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 RNA 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 the "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.

Always use filter barrier pipette tips to prevent cross contamination

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

Recipe

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

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 cDNA derived from RNA by the use of reverse transcriptase.
RT-PCR mix enzymes are available commercially that can be substituted for Taq Polymerase in the following protocol.
The HCV Genemer™ amplifies a 214 bp fragment.

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

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

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

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).

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.

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

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	1 µl	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		

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

1X 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 214 bp represents specific amplification from *Hepatitis C Virus* RNA. It will be present in samples having *Hepatitis C Virus* RNA template.

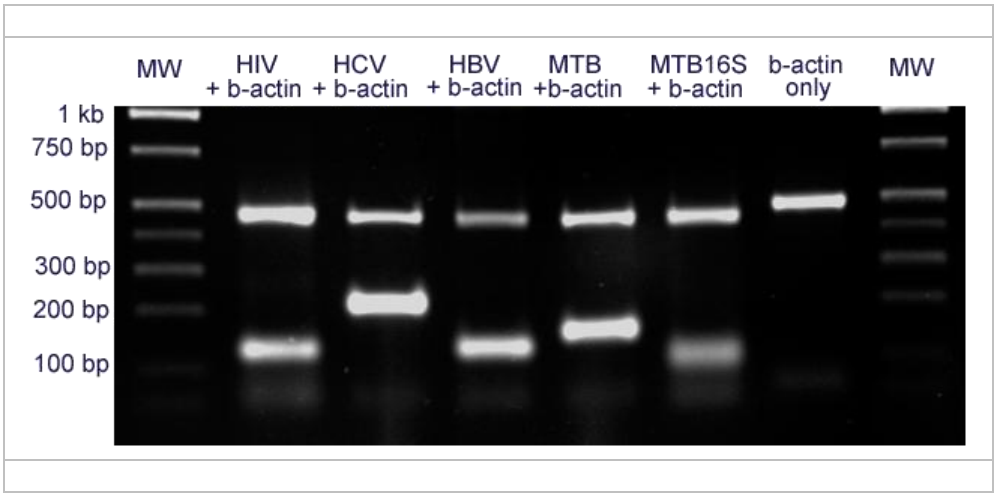
1. Two bands seen in the gel (500 bp and 214 bp):

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 the *Hepatitis C Virus* is used to identify an HCV 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, Bacillar 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, or otherwise stored at –20°C for RNA 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. Kontorinis N, Agarwal K, Dieterich DT Current status of the use of growth factors and other adjuvant medications in patients receiving peginterferon and ribavirin. Rev Gastroenterol Disord. 2004;4 Suppl 1:S39-47
2. Dal Molin G, Tiribelli C, Campello C. A rational use of laboratory tests in the diagnosis and management of hepatitis C virus infection. Ann Hepatol. 2003 Apr-Jun;2(2):76-83
3. Carey W. Tests and screening strategies for the diagnosis of hepatitis C. Cleve Clin J Med. 2003 Sep; 70 Suppl 4:57-13
4. Sarrazin C. Highly sensitive hepatitis C virus RNA detection methods: molecular backgrounds and clinical significance. J Clin Virol. 2002 Dec; 25 Suppl 3:S23-9.

Appendix

Pathogen RNA Purification

1. Transfer 50 µl of serum to a microfuge tube. Add 250 µl of RNA 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 RNA 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 RNA wash buffer and vortex for 1 minute.
8. Centrifuge at 5,000 rpm for 1 minute at RT. Discard supernatant and add 35 µl of RNase free water and vortex for 1 minute.
9. Centrifuge at 5,000 rpm for 1 minute at RT to elute purified RNA.
10. Use 8 µl of purified RNA for RT-PCR reaction.
11. The eluted RNA 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.

First Strand cDNA Synthesis

First strand cDNA synthesis classically is performed by using a reverse transcriptase. The cDNA thus obtained can be used as the starting material for the PCR amplification.
The above two-step procedure can be combined and is termed as RT-PCR (reverse transcriptase-polymerase chain reaction). This is a widely used and powerful method of amplifying first strand cDNA products by the polymerase chain reaction.

Various commercial RT-PCR systems are available.

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.