Product Manual

HIV-1 Genemer™ Mix

Primer Pair for amplification of HIV-1 Specific DNA Fragment
Includes Internal Negative Control Primers and Template

Catalog No.: 60-2002-12 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

Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Mix

Primer Pair for amplification of HIV-1 Specific DNA Fragment
Includes Internal Negative Control Primers and Template

Catalog No.: 60-2002-12 **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)
HIV 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.	60-2002-12	200 rxns

Introduction

The Human Immunodeficiency Virus (HIV) is the causative agent of Acquired Immune Deficiency Syndrome (AIDS). There are two types of HIV virions responsible for human infections: HIV-1 and HIV-2. Which differ in their virulence and prevalence. Most reported cases of AIDS around the world have been attributed to HIV-1. The virus destroys a certain type of blood cell, known as T-cells or a CD4 cell, which helps the body, fight off infections. A person can be infected with HIV for many years before any symptoms occur, and during this time, an infected person can unknowingly pass the infection on to others. HIV can be transmitted by sexual contact, mother-to-child transmission (MTCT) during pregnancy, delivery, or breastfeeding, and by contact with infected blood and other body fluids. Examples of this are the use of shared needles, the use of contaminated skin-cutting tools, needle stick injuries in health care settings, or transfusion of infected blood.

At an advanced stage of HIV infection that occurs when the immune system cannot fight off opportunistic infections that the body is normally able to withstand. At this stage, the infected person increasingly develops symptoms triggered by such infections. Some examples include chronic cryptosporida diarrhea, cytomegalovirus eye infection, pneumocystis pneumonia, toxoplasmosis, and tuberculosis as well as infections with members of the Mycobacterium avium complex. In addition the development of different types of cancer, such as invasive cervical cancer, Kaposi sarcoma or lymphoma, is frequently observed. At present, there is no cure for AIDS, and it is believed that most people with HIV infection will eventually die of an AIDS-related illness. However, advancements in HIV/AIDS therapies, including those that fight the virus itself as well as those that prevent and treat opportunistic infections, have drastically improved life expectancy and quality for many HIV/AIDS patients.

Diagnostic tests for HIV infection utilize four approaches: detection of HIV-1 antibodies, detection of viral antigens, detection of viral nucleic acids and culturing the virus. Most cases of HIV are diagnosed by testing the patient's serum for antibodies against HIV-1, using an enzyme-linked immunosorbent assay (ELISA, EIA). This ELISA test is highly sensitive and specific, but both false-positive and false-negative tests can occur. The median time from initial HIV exposure to the development of detectable antibodies is two months, with 95% of persons developing antibodies within six months. Therefore, false-negatives may occur if the patient was recently infected and has not yet sero-converted. Positive ELISA results are repeated, and if the repeated assay is also positive, then a confirmatory test is ordered, usually a Western blot or indirect immuno-fluorescence assay (IFA). These confirmatory tests also detect the presence of anti-HIV antibodies in serum but are more specific, determining the specific antigen against which the antibody is directed.

In recent years, new molecular techniques have been developed that allow plasma HIV RNA levels (viral load) to be measured. Viral load directly quantifies the amount of virus-producing cells in the plasma throughout the course of disease. This important surrogate marker has emerged as a valuable tool for determining when to initiate antiretroviral therapy, for predicting disease progression and for monitoring response to drug therapy. Recent studies have shown that plasma HIV RNA levels are strong predictors of disease progression. Higher HIV RNA levels (i.e., greater than 100,000 copies/ml) are associated with a more rapid decline in CD4+ lymphocyte counts and a more rapid progression to AIDS. Viral load is generally expressed as the number of viral RNA copies per milliliter of plasma.

Gene Link Diagnostics gene detection system uses the Polymerase Chain Reaction (PCR) to amplify the amount of a specific gene fragment DNA or RNA present in a sample. Amplification of RNA virus templates is based on reverse transcriptase PCR (RTPCR). The method is highly sensitive and is capable of detecting a few copies of template. Detection systems offered by Gene Link Diagnostics 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 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 "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.

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

Amplification and Detection

This protocol assumes that amplification is being performed from purified DNA.
The HIV-1 Genemer™ amplifies a 125 bp fragment.

Program your thermal cycler instrument with an amplification profile prior to starting 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.

HIV Amplification Profile		
Step	**Temperature & Time**	**Cycles**
Initial Denaturation	5 min at 95°C	1
Denaturation	15 seconds at 94°C	30
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

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		

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

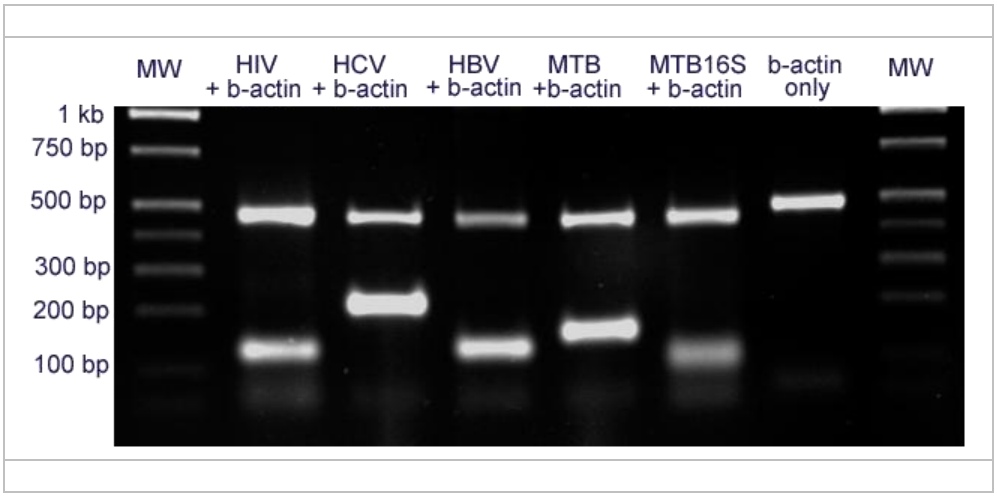
Primers for negative internal control are included to verify faithful amplification protocol. The negative internal control amplification fragment is of ~500 bp and should be seen in all amplification reactions. The lower fragment of ~125 bp represents specific amplification from Human Immunodeficiency Virus-1 (HIV-1) DNA. It will be present in samples having Human Immunodeficiency Virus-1 (HIV-1) DNA template.

1. Two bands seen in the gel (500 bp and 125 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



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 DNA.
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. Uphold CR, Maruenda J, Yarandi HN, Sleasman JW, Bender BS. HIV and older adults: clinical outcomes in the era of HAART.
   J Gerontol Nurs. 2004 Jul; 30(7): 16-24; quiz 55-6
2. Rakhmanina NY, van den Anker JN, Soldin SJ. Therapeutic drug monitoring of antiretroviral therapy. AIDS Patient Care STDS. 2004 Jan; 18(1): 7-14
3. Peter JB, Sevall JS. Molecular-based methods for quantifying HIV viral load. AIDS Patient Care STDS. 2004 Feb; 18(2): 75-9
4. Volberding PA. Initiating HIV therapy. Timing is critical, controversial. Postgrad Med. 2004 Feb; 115(2): 15-8, 21, 24-6.

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.