Product Manual

Mycobacterium tuberculosis Genemer™ Amplification Kit

Amplification premix for MTB Viral Specific Fragment

Catalog No.: 60-2004-11K Store at –20°C

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

- **All human and animal samples, bodily fluids including purified DNA & RNA for pathogen detection should be considered infectious.**
- **Appropriate protective gear (gloves, mask, goggles and disposable laboratory coat) and decontamination protocol should be followed.**
- **All waste should be decontaminated prior to disposal.**
- **Follow approved laboratory, state and local regulations for decontamination protocol and disposal of all samples and laboratory ware.**
- **See appendix for an example of decontamination protocol.**

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 who are appropriately trained in performing molecular biology techniques following established safety procedures. Additional qualification and certification is required for interpretation of results.

Material supplied

Mycobacterium tuberculosis (MTB) Genemer™ Amplification Kit

Ready-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. Ready to use; simply add sample and Taq Polymerase. The kit includes nuclease free water and 5X Orange G/XC loading buffer.

Storage Instructions

1. Shipped on ice.
2. Store at –20°C upon receipt.

	Qty	Catalog No.	Product	Size
❑		**60-2004-11KL**	**Mycobacterium tuberculosis (MTB) Genemer™ Amplification Kit; 100 Reactions**	**100 Reactions**
❑	1	60-2004-11L	Mycobacterium tuberculosis (MTB) Genemer™ PCR Premix 1 mL	100 Reactions
❑	1	60-2004-06C	Mycobacterium tuberculosis (MTB) Genemer™ Control DNA 20 µL (1 ng/µL)	20 µL
❑	1	40-3001-01	Nuclease Free Water	1 mL
❑	1	40-3004-05	Loading buffer 5X Orange G/XC non-denaturing	0.5 mL

	Qty	Catalog Number	Product	Size
❑		**60-2004-11KS**	**Mycobacterium tuberculosis (MTB) Genemer™ Amplification Kit; 50 Reactions**	**50 Reactions**
❑	1	60-2004-11S	Mycobacterium tuberculosis (MTB) Genemer™ PCR Premix 0.5 mL	50 Reactions
❑	1	60-2004-06C	Mycobacterium tuberculosis (MTB) Genemer™ Control DNA 20 µL (1 ng/µL)	20 µL
❑	1	40-3001-01	Nuclease Free Water	1 ml
❑	1	40-3004-05	Loading buffer 5X Orange G/XC non-denaturing	0.5 ml

*The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Introduction

TB, or tuberculosis, is a disease caused by bacteria called *Mycobacterium tuberculosis* (MTB). The bacteria can attack any part of your body, but they usually attack the lungs. TB disease was once the leading cause of death in the United States.

Tuberculosis is a chronic, cyclic disease, mainly affecting the lung and the associated lymph nodes. But depending on the immune status of the patient, the *Mycobacterium tuberculosis* bacteria can also colonize other organs. TB gets spread from person to person via aerosols. Only people with active disease are contagious. Especially in the immunosuppressed people the *Mycobacterium tuberculosis* can be reactivated and be spread over the whole body, even years after the initial infection.

TB is spread through the air from one person to another. The bacteria are put into the air when a person with TB disease of the lungs or throat coughs or sneezes. People nearby may breathe in these bacteria and become infected. People who are infected with latent TB do not feel sick, do not have any symptoms, and cannot spread TB. But they may develop TB disease at some time in the future. People with TB disease can be treated and cured if they seek medical help. Even better, people who have latent TB infection but are not yet sick can take medicine so that they will never develop TB disease.

Tuberculosis (TB) is still one of the most infectious diseases worldwide. Some two billon people, one-third of the world's population, are infected with *Mycobacterium tuberculosis*, the causative agent of tuberculosis. The incidence of tuberculosis worldwide is about eight million and about three million people die each year. The largest number of cases occurs in the Third World Countries. But tuberculosis is a reemerging disease in the industrialized nations, mainly due to the immigration of infected people and the development of drug resistant TB. Minorities like homeless, drug users and immune compromised persons are affected disproportionately.

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 (RTPCR). 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

- All human and animal samples, bodily fluids including purified DNA & RNA for pathogen detection should be considered infectious.
- Appropriate protective gear (gloves, mask, goggles and disposable laboratory coat) and decontamination protocol should be followed.
- All waste should be decontaminated prior to disposal.
- Follow approved laboratory, state and local regulations for decontamination protocol and disposal of all samples and laboratory ware.
- See appendix for an example of decontamination protocol.

Genemer™ Amplification Premix

The Genemer™ Amplification premix is supplied as a 2X concentrated ready to use reagent. Keep on ice and immediately freeze after use. Simply add template and Taq polymerase.

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 cDNA/DNA.

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

Always use filter barrier pipette tips to prevent cross contamination

Stringent laboratory conditions should be followed to avoid cross contamination. Generally a few copies of template are sufficient for a successful amplification.

Amplification Profile		
Step	**Temperature & Time**	**Cycles**
Initial Denaturation	5 min at 95°C	1
Denaturation	15 seconds at 94°C	40
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

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

1. PCR* Setup

It is a good strategy initially to amplify multiple amounts for optimization. Use known positive control DNA as one sample. Genemer™ positive control template is provided in this kit. Use 2 µl of this control DNA as one sample. Use one amplification reaction as blank with no added template DNA to determine background amplification due to contamination. The blank reaction should have no amplification product.

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 Setup		
Component	**1 X 20 µl Rxn.**	**10 X 20 µl Rxns.**
Sterile Water	Up to 8 µl	Up to 80 µl
2X Genemer™ PCR Premix	10 µl	100 µl
Taq DNA Polymerase Enzyme or Enzyme mix (EM)	0.5 µl	3 µl
Template DNA	1-5 µl	Add 1-5 µl DNA to each tube
Total Volume	20 µl	
Keep on ice during set up. After adding template start PCR File		

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		**10 X 20 µl Rxns.**
Sterile Water		33 µl
10 X PCR Buffer		4 µl
Taq Polymerase		3 µl
Add 3 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.

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

Prepare a 2 % agarose gel containing 1 µg/ml ethidium bromide in TAE buffer.

1. Add 2 µl of 5X 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.

Visualize under UV. Obtain documentation by taking a gel picture.

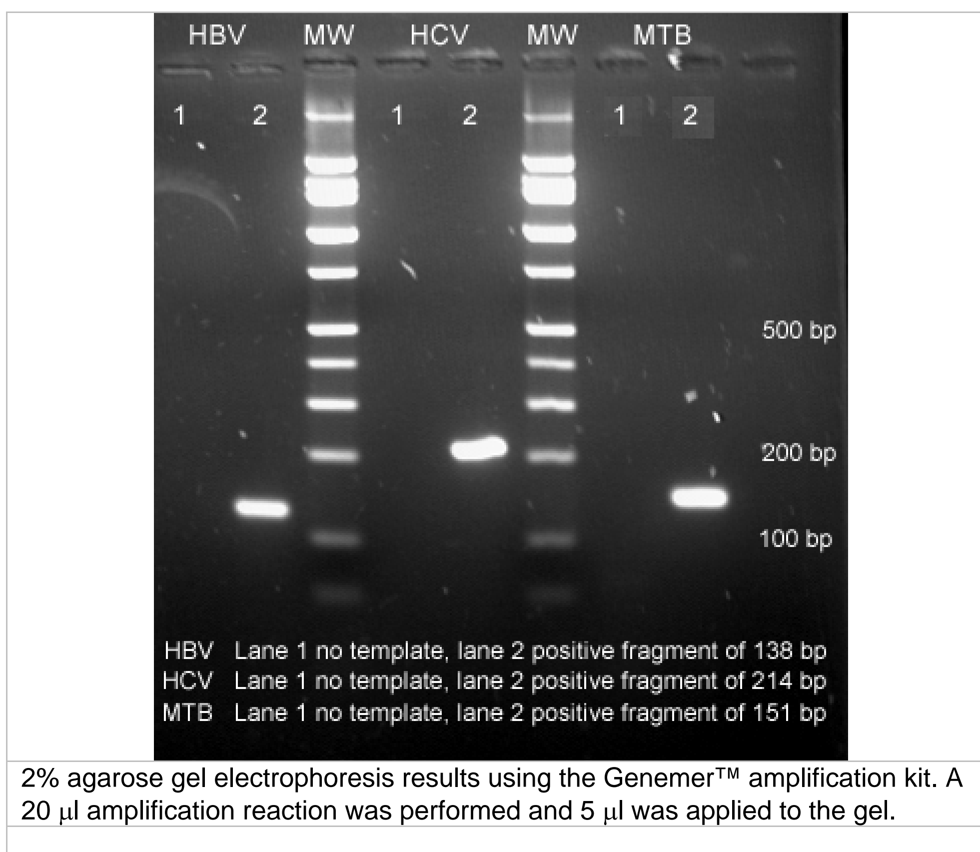
Recipe

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

Refer to the table below to determine the expected size of amplified fragment.

Pathogen Genemer™ Expected Fragment Size		
Pathogen	Control DNA Catalog No.	Fragment Size*
WNV	60-2001-06	154 bp
HIV	60-2002-06	125 bp
HCV	60-2003-06	214 bp
MTB	60-2004-06	151 bp
HBV	60-2007-06	138 bp
MTB 16s RNA	60-2012-06	175 bp
*Fragment size obtained after PCR amplification		



2% agarose gel electrophoresis results using the Genemer™ amplification kit. A 20 μl amplification reaction was performed and 5 μl was applied to the gel.

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 ^{o}C for DNA and –70 ^{o}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. Reischl U, Lehn N, Wolf H, Naumann L Clinical evaluation of the automated COBAS AMPLICOR MTB assay for testing respiratory and nonrespiratory specimens. J Clin Microbiol. 1998 Oct; 36(10):2853-60
2. Laifer G, Widmer AF, Frei R, Zimmerli W, Fluckiger U Polymerase chain reaction for Mycobacterium tuberculosis: impact on clinical management of refugees with pulmonary infiltrates. Chest. 2004 Mar; 125(3): 981-6.
3. Wan GH, Lu SC, Tsai YH. Polymerase chain reaction used for the detection of airborne Mycobacterium tuberculosis in health care settings. Am J Infect Control. 2004 Feb;32(1):17-22.
4. Jonsson B, Ridell M. The Cobas Amplicor MTB test for detection of Mycobacterium tuberculosis complex from respiratory and non-respiratory clinical specimens. Scand J Infect Dis. 2003;35(6-7):372-7.

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.

Appendix

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 & Laboratory Plastic Disposables

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 plastic ware 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

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*	Price $
60-2001-10	West Nile Virus (WNV) Genemer™	2 nmols	60.00
60-2002-10	Human Immunodeficiency Virus-1 (HIV-1) Genemer™	2 nmols	60.00
60-2003-10	Hepatitis C Virus (HCV) Genemer™	2 nmols	60.00
60-2004-10	Mycobacterium tuberculosis (MTB) Genemer™	2 nmols	60.00
60-2007-10	Hepatitis B Virus (HBV) Genemer™	2 nmols	60.00
*The quantity supplied is sufficient for 200 regular 20 µl PCR reactions			

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

Genemer™ Amplification Kit: Ready-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. Ready to use; simply add sample and Taq Polymerase. The kit includes sterile water and 5X Orange G/XC loading buffer.

Catalog No.	Product	Size	Price $
60-2001-11KL	West Nile Virus (WNV) Genemer™ Amplification Kit; 100 detections (1 ml)	100 Rxns	140.00
60-2001-11KS	West Nile Virus (WNV) Genemer™ Amplification Kit; 50 detections (0.5 ml)	50 Rxns	80.00
60-2002-11KL	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Amplification Kit; 100 detections (1 ml)	100 Rxns	140.00
60-2002-11KS	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Amplification Kit; 50 detections (0.5 ml)	50 Rxns	80.00
60-2003-11KL	Hepatitis C Virus (HCV) Genemer™ Amplification Kit; 100 detections (1 ml)	100 Rxns	140.00
60-2003-11KS	Hepatitis C Virus (HCV) Genemer™ Amplification Kit; 50 detections (0.5 ml)	50 Rxns	80.00
60-2004-11KL	Mycobacterium tuberculosis (MTB) Genemer™ Amplification Kit; 100 detections (1 ml)	100 Rxns	140.00
60-2004-11KS	Mycobacterium tuberculosis (MTB) Genemer™ Amplification Kit; 50 detections (0.5 ml)	50 Rxns	80.00
60-2007-11KL	Hepatitis B Virus (HBV) Genemer™ Amplification Kit; 100 detections (1 ml)	100 Rxns	140.00
60-2007-11KS	Hepatitis B Virus (HBV) Genemer™ Amplification Kit; 50 detections (0.5 ml)	50 Rxns	80.00
*20 µl PCR reaction volume			

*Please visit www.genelink.com for other Genemer™ Control DNA 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	Price $
60-2001-06	West Nile Virus (WNV) Genemer™ Control DNA	500 ng	175.00
60-2002-06	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Control DNA	500 ng	175.00
60-2003-06	Hepatitis C Virus (HCV) Genemer™ Control DNA	500 ng	175.00
60-2004-06	Mycobacterium tuberculosis (MTB) Genemer™ Control DNA	500 ng	175.00
60-2007-06	Hepatitis B Virus (HBV) Genemer™ Control DNA	500 ng	175.00

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