Product Manual

Mycobacterium tuberculosis Genemer™

Primer Pair for amplification of MTB DNA Specific Fragment

Catalog No.: 60-2004-10 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

Mycobacterium tuberculosis Genemer™
Primer Pair for amplification of MTB Specific DNA Fragment

Catalog No.: 60-2004-10 **10 nmols**

Each tube contains 10 nmols lyophilized primer Genemer™ pair. The quantity supplied is sufficient for 800 regular 25 µl PCR reaction.

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
Mycobacterium tuberculosis Genemer™ Primer Pair for amplification of MTB Specific DNA Fragment.	60-2004-10	10 nmols

*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

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.

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™ Reconstitution

Stock Primer Mix: Dissolve the supplied lyophilized Genemer™ in 100 µl sterile TE. The 10 nmols of primers when dissolved in 100 µl will give a solution of 100 µM i.e. 100 pmols/µl.

Primer Mix: Prepare a 10 pmols/µl Primer Mix solution by a ten fold dilution of the stock primer mix. Example: Add 180 µl sterile TE to a new tube, to this tube add 20 µl of primer stock solution. Label this tube as Primer Mix 10 pmols/µl.

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 MTB Genemer™ amplifies a 138 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.

MTB 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
10 pmol/µl Primer 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

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.

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 ~200 bp represents specific amplification from *Mycobacterium Tuberculosis* DNA. It will be present in samples having *Mycobacterium Tuberculosis* DNA template.

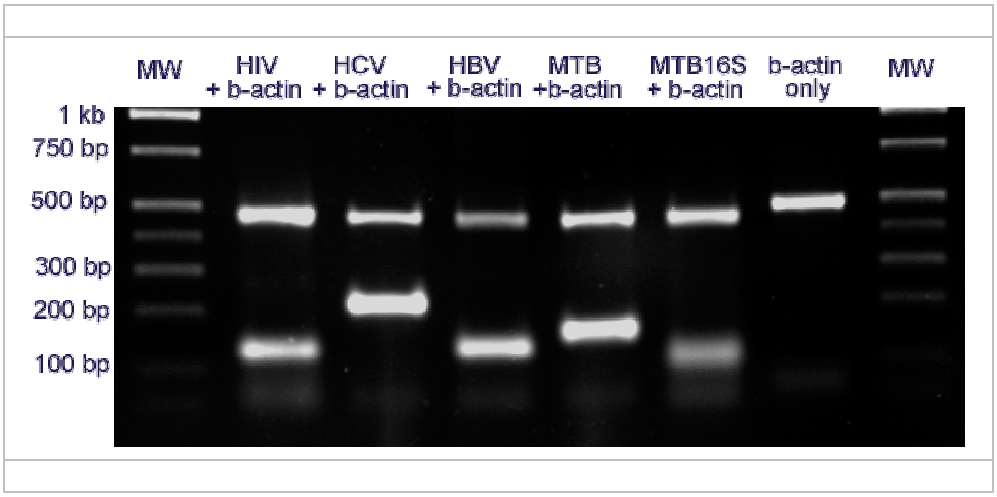
1. Two bands seen in the gel (500 bp and 200 bp):

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 *Mycobacterium Tuberculosis* is used to identify an MTB 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, 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. 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 Mycobacterium tuberculosis 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 MTB assays systems. Methods Mol Med. 2004; 95:3-13. Review.

4. Hu KQ. Occult Mycobacterium tuberculosis infection and its clinical implications. J Viral Hepat. 2002 Jul;9(4):243-57.

Appendix

Pathogen DNA Extraction

1. Add an equal volume of 2% (wt/vol) NaOH-1.45%(wt/vol) trisodium citrate $2H_2O$ to the sputum/BAL. Starting volume should not exceed 2 ml.
2. Vortex for 1 minute and incubate at RT for 20 minutes.
3. Centrifuge at 10,000 rpm for 15 minutes at RT.
4. Carefully decant the supernatant.
5. Add 250 µl of DNA lysis buffer and 10 µl of Glass Beads. Vortex for 1 minute and incubate at RT for 5 minutes.
6. Transfer all the contents into an elution tube. Incubate at 65°C for 15 minutes.
7. Incubate at 95°C for 5 minutes.
8. Incubate at room temperature (RT) for 15 minutes.
9. Centrifuge at 5,000 rpm for 1 minute at RT.
10. 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.
11. 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.
12. Centrifuge at 5,000 rpm for 1 minute at RT.
13. Discard supernatant and add 35 µl of RNase free water and vortex for 1 minute.
14. Centrifuge at 5,000 rpm for 1 minute at RT to elute purified DNA.
15. Use eluted DNA for the PCR reaction.
16. 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 DNA purification protocol is specific for the Omni-Pure ™ Microbial DNA Purification System; Gene Link catalog No: 40-3700-XX. Please refer to product manual for detailed information and protocol.

Other reliable purification protocol and or product can be substituted.

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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™	10 nmols	100.00
60-2002-10	Human Immunodeficiency Virus-1 (HIV-1) Genemer™	10 nmols	100.00
60-2003-10	Hepatitis C Virus (HCV) Genemer™	10 nmols	100.00
60-2004-10	Mycobacterium tuberculosis (MTB) Genemer™	10 nmols	100.00
60-2007-10	Hepatitis B Virus (HBV) Genemer™	10 nmols	100.00
*The quantity supplied is sufficient for 800 regular 25 µl PCR reactions			

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

Genemer™ Detection 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	Price $
60-2001-11	West Nile Virus (WNV) Genemer™ Kit; 100 detections	1 Kit	250.00
60-2001-11S	West Nile Virus (WNV) Genemer™ Kit; 50 detections	1 Kit	150.00
60-2002-11	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Kit; 100	1 Kit	250.00
60-2002-11S	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Kit; 50 detections	1 Kit	150.00
60-2003-11	Hepatitis C Virus (HCV) Genemer™ Kit; 100 detections	1 Kit	250.00
60-2003-11S	Hepatitis C Virus (HCV) Genemer™ Kit; 50 detections	1 Kit	150.00
60-2004-11	Mycobacterium tuberculosis (MTB) Genemer™ Kit; 100 detections	1 Kit	250.00
60-2004-11S	Mycobacterium tuberculosis (MTB) Genemer™ Kit; 50 detections	1 Kit	150.00
60-2007-11	Hepatitis B Virus (HBV) Genemer™ Kit; 100 detections	1 Kit	250.00
60-2007-11S	Hepatitis B Virus (HBV) Genemer™ Kit; 50 detections	1 Kit	150.00

*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*	Price $
60-2001-12	West Nile Virus (WNV) Genemer™ Mix	200 reactions	100.00
60-2002-12	Human Immunodeficiency Virus-1 (HIV-1) Genemer™ Mix	200 reactions	100.00
60-2003-12	Hepatitis C Virus (HCV) Genemer™ Mix	200 reactions	100.00
60-2004-12	Mycobacterium tuberculosis (MTB) Genemer™ Mix	200 reactions	100.00
60-2007-12	Hepatitis B Virus (HBV) Genemer™ Mix	200 reactions	100.00
*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	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

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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	Price $
40-4010-01	Omni-Pure™ Genomic DNA Purification System	100	75.00
40-4010-05	Omni-Pure™ Genomic DNA Purification System	500	210.00
40-4010-10	Omni-Pure™ Genomic DNA Purification System	1000	350.00
40-3720-01	Omni-Pure™ Viral DNA Purification System	100	220.00
40-3720-05	Omni-Pure™ Viral DNA Purification System	500	880.00
40-3720-50	Omni-Pure™ Viral DNA Purification System	1000	1400.00
40-3700-01	Omni-Pure™ Microbial DNA Purification System	100	175.00
40-3700-05	Omni-Pure™ Microbial DNA Purification System	500	700.00
40-3700-10	Omni-Pure™ Microbial DNA Purification System	1000	1120.00
40-3650-01	Omni-Pure™ Viral RNA Purification System	100	175.00
40-3650-05	Omni-Pure™ Viral RNA Purification System	500	700.00
40-3650-10	Omni-Pure™ Viral RNA Purification System	1000	1120.00
40-4110-10	Omni-Clean™ Gel DNA Beads Purification System	100	95.00
40-4110-50	Omni-Clean™ Gel DNA Beads Purification System	500	380.00
40-4120-10	Omni-Clean™ Gel DNA Spin Column Purification System	100	110.00
40-4120-50	Omni-Clean™ Gel DNA Spin Column Purification System	500	440.00
40-4130-10	Omni-Clean™ DNA Beads Concentration System	100	95.00
40-4130-50	Omni-Clean™ DNA Beads Concentration System	500	380.00
40-4140-10	Omni-Clean™ DNA Spin Column Concentration System	100	110.00
40-4140-50	Omni-Clean™ DNA Spin Column Concentration System	500	440.00

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	Price $
40-3005-01	Omni-Marker™ Universal unlabeled	100 µl	15.00
40-3005-05	Omni- Marker™ Universal unlabeled	500 µl	50.00
40-3005-10	Omni-Marker™ Universal unlabeled	1 ml	90.00
40-3006-01	Omni- Marker™ Low unlabeled	100 µl	15.00
40-3006-05	Omni-Marker™ Low unlabeled	500 µl	50.00
40-3006-10	Omni- Marker™ Low unlabeled	1 ml	90.00
40-3062-01	Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	100 µl	75.00
40-3062-05	Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	500 µl	325.00
40-3002-01	Loading Buffer 5X BPB/XC non-denaturing	100 µl	5.00
40-3002-10	Loading Buffer 5X BPB/XC non-denaturing	1 ml	10.00
40-3004-01	Loading Buffer 5X Orange G/XC non-denaturing	100 µl	5.00
40-3004-10	Loading Buffer 5X Orange G/XC non-denaturing	1 ml	10.00
40-5027-01	Loading Buffer 2X BPB/XC Denaturing for Sequencing	100 µl	5.00
40-5027-10	Loading Buffer 2X BPB/XC Denaturing for Sequencing	1 ml	10.00
40-5020-20	Hybwash A, Hybridization Wash Solution	200 ml	65.00
40-5021-10	Hybwash B, Hybridization Wash Solution	100 ml	50.00
40-5025-20	10x Washing buffer	200 ml	125.00
40-5026-10	10% Blocking solution	100 ml	75.00
40-5027-00	Seq. Loading buffer	1 ml	10.00
40-5031-10	10x AP Detection buffer	100 ml	65.00
40-5022-20	Lumisol™ I Hybridization Solution; contains formamide	200 ml	75.00
40-5023-20	Lumisol™ II Hybridization Solution; for non-toxic hybridizations	200 ml	75.00
40-5024-20	Lumisol™ III Hybridization Solution; for oligo probes	200 ml	75.00

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