

October 2016

bactotype[®] Mastitis HP3 PCR Kit Handbook



96 (catalog no. 280045)

For detection of DNA from *Streptococcus agalactiae*, *Mycoplasma bovis* and *Staphylococcus aureus*

REF

280045



QIAGEN Leipzig GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

Contents

Kit Contents	3
Intended Use.....	3
Symbols	3
Storage	4
Safety Information	4
Quality Control	5
Introduction	6
Principle.....	7
DNA extraction	8
Equipment and Reagents to Be Supplied by User	9
Important Notes	10
General precautions.....	10
Negative control	10
Positive control.....	10
Internal control.....	11
Protocol: Real-Time PCR for Detection of Mastitis-Causing Pathogens.....	12
Data Analysis and Interpretation	15
Troubleshooting Guide	18
Ordering Information	19

Kit Contents

<i>bactotype</i> Mastitis HP3 PCR Kit	(96)
Catalog no.	280045
Number of reactions	96
Master Mix (tube with orange cap), includes enzymes, primers and probes	2 x 980 µl
Positive Control (tube with red cap)	1 x 100 µl
Negative Control (tube with blue cap)	1 x 100 µl
Handbook	1

Intended Use

The *bactotype* Mastitis HP3 PCR Kit is intended for the detection of DNA from *Streptococcus agalactiae*, *Mycoplasma bovis* and *Staphylococcus aureus* in ruminant milk.

For veterinary use only.

Symbols



<N>

Contains reagents for <N> tests



Legal manufacturer

	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
	Catalog number
	Material number
	Protect from light
	For ruminant milk samples

Storage

The components of the *bactotype* Mastitis HP3 PCR Kit should be stored at -30°C to -15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing ($>2\times$), as this may reduce assay sensitivity. Freeze the components in aliquots if they will only be used intermittently.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information,

please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infective material.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *bactotype* Mastitis HP3 PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *bactotype* Mastitis HP3 PCR Kit is a highly sensitive and specific solution for the detection of DNA from three major mastitis-causing pathogens *Streptococcus agalactiae*, *Mycoplasma bovis* and *Staphylococcus aureus* in samples from ruminant milk.

Bovine mastitis is the single most costly disease of dairy cattle worldwide, mainly caused by intra-mammary infection (IMI). This may be associated with increased somatic cell counts in bovine milk and a reduction of milk yield and quality.

Mastitis manifests itself as clinical or sub-clinical mastitis. Clinical mastitis can be further classified as mild, moderate or severe/fatal and as chronic mastitis, which often require antimicrobial therapy.

A wide range of bacteria can cause mastitis and can be subdivided into cow-associated (or contagious mastitis) pathogens (e.g., *S. agalactiae*, *M. bovis*, *S. aureus*) and environmental pathogens (e.g., *S. uberis*, *E. coli*, *Klebsiella*).

The *bactotype* Mastitis HP3 PCR Kit detects the most important cow-associated and highly contagious bacteria *S. agalactiae*, *M. bovis* and *S. aureus* with high sensitivity.

It allows the detection of these pathogens in quarter milk samples as well as in pool or bulk milk.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is detected using fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows detection of the accumulating product without the need to re-open the reaction tubes afterward.

The *bactotype* Mastitis HP3 PCR Kit contains all of the necessary reagents for the simultaneous detection of DNA from *S. agalactiae*, *M. bovis* and *S. aureus*, including positive and negative controls.

An internal control excludes the possibility of false-negative results.

The kit uses four specific primer/probe combinations: one for *S. aureus* DNA, yielding FAM™ fluorescence; one for *S. agalactiae*, yielding JOET™ fluorescence; one for *M. bovis*, yielding Cy®5 fluorescence; and one for the internal control (β -actin DNA) present within the sample, yielding Texas Red fluorescence. A positive control serves to verify the functionality of the reaction mix for the amplification of one of the DNA targets per channel.

DNA extraction

The *bactotype* Mastitis HP3 PCR Kit can be used for the detection of pathogen DNA from ruminant milk samples. Due to the high sensitivity of the test, individual quarter milk samples, pool milk samples or tank milk samples can be used.

Prior to real-time PCR, bacterial DNA must be extracted from the starting material. QIAGEN recommends using the following products for DNA extraction from milk samples:

- DNeasy® Mastitis Mini Kit (192), cat. no. 69805
- MagAttract® Mastitis Kit (384), cat. no. 947757

If real-time PCR is not performed immediately after extraction, store the DNA at -30°C to -15°C .

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Pipets
- Nuclease-free aerosol-resistant pipet tips with filters
- Sterile 1.5 ml Eppendorf® tubes
- Nuclease-free (RNase/DNase-free) consumables. Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive identification of nucleic acids
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Cooling device or ice
- 96-well plate real-time cycler with appropriate fluorescent channels (e.g., Agilent® Mx3005P® or Bio-Rad® CFX96)
- Appropriate software for the chosen 96-well plate cycler
- 96-well optical microplate with optical sealing film or cover for the chosen 96-well plate real-time cycler

Important Notes

General precautions

The user should always pay attention to the following:

- Use nuclease-free pipet tips with filters.
- Store and extract positive materials (specimens, positive controls and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components on ice before starting an assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit after the expiration date.
- Keep samples and controls on ice or in a cooling block during setup of reactions.

Negative control

At least one negative control reaction should be included in each PCR run. This enables assessment of contamination in the reaction.

Positive control

When performing PCR on unknown samples, it is recommended to perform a positive control reaction in the PCR run, containing a sample that is known to include the targeted bacterial DNA. A

positive control serves to prove the functionality of the pathogen assay, for example, the correct setup of the reaction mix. Use 5 μ l of the positive control provided in the *bactotype* Mastitis HP3 PCR Kit to test for successful amplification of the target.

Internal control

For increased process safety and convenience, an internal control assay is included in the form of an additional primer/probe set that detects a housekeeping gene (β -actin DNA) present within the sample. This allows monitoring of both extraction and amplification.

Protocol: Real-Time PCR for Detection of Mastitis-Causing Pathogens

Important points before starting

- Read “Important Notes”, page 10, before starting.
- Include at least one positive control (Positive Control) and one negative control (Negative Control) per PCR run.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- Perform the protocol without interruption.

Things to do before starting

- Thaw all reagents on ice and protect from light.
- Maintain reagents on ice during PCR setup.
- Before use, centrifuge the reagents briefly.

Procedure

1. Pipet 20 μ l of the Master Mix into each reaction tube. Then add 5 μ l of the sample DNA (Table 1, page 13).

Include positive and negative control reactions.

Positive control: Use 5 μ l of the positive control (Positive Control) instead of sample DNA.

Negative control: Use 5 μ l of the negative control (Negative Control) instead of sample DNA.

Table 1. Preparation of reaction mix

Component	Volume
Master Mix	20 μ l
Sample	5 μ l
Total volume	25 μl

2. Close the reaction tubes with the corresponding caps.
3. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for reporter

Pathogen/internal control	Reporter
<i>S. aureus</i>	FAM
<i>S. agalactiae</i>	HEX/JOE*
<i>M. bovis</i>	Cy5
Internal control	Texas Red/ROX*

* Use the option appropriate for your thermal cycler. For ABI 7500 and Agilent Mx3005P, do not use ROX as Passive Reference or Reference dye.

4. Run the real-time PCR protocol according to Table 3.

Table 3. Real-time PCR protocol for *bactotype* Mastitis PCR Kits

Temperature	Time	Number of cycles
95°C	5 min	1
95°C	10 s	40
57°C*	30 s	

* Fluorescence data collection.

(Approximate run time on the Agilent Mx3005P is 65 min.)

Data Analysis and Interpretation

For the assay to be valid:

- The positive control yields a signal in all four channels (FAM, HEX/JOE, Texas Red/ROX and Cy5) with a C_T^* <35.
- The negative control does not yield a signal in any of the four channels.

For interpretation of the sample results:

- The results summarized in Table 4, page 17, are possible if working with unknown samples.
- Check that the sample yields an internal control signal in the Texas Red/ROX channel. A positive Texas Red/ROX signal means that extraction and amplification were successful since the housekeeping gene within the sample is amplified.
- The sample is negative if a signal is detected only in the Texas Red/ROX channel.
- If no signal is detected in all channels (FAM, HEX/JOE, Texas Red/ROX and Cy5), the result is inconclusive, and the assay is invalid. The absence of a signal for the housekeeping gene indicates PCR inhibition and/or other malfunctions, such as extraction failure.

To check for inhibition, we recommend diluting the sample DNA 1:5 in nuclease-free water, repeating the DNA extraction, or repeating the whole test procedure starting with new sample material.

* Threshold cycle (C_T) is the cycle at which the amplification plot crosses the threshold, i.e., where there is the first clearly detectable increase in fluorescence.

Check that there is a fluorescence signal in the pathogen channels (FAM, HEX/JOE, and Cy5) for the positive control reaction (Positive Control). Absence of a signal for the positive control indicates an error, which could be due to incorrect setup of the reaction mix or incorrect cycling conditions.

Internal control

The assay internal control serves as an extraction and amplification control and is based on a ruminant housekeeping gene. Amplification of the internal control means that nucleic acid extraction and target DNA amplification from milk was successful.

As additional information, the C_T value of the internal control can provide an indication of the amount of somatic cells in the milk sample. A low C_T value of the internal control ($C_T < 29$) may suggest a high somatic cell count in the milk sample.

A low internal control C_T value in the absence of a positive signal in the pathogen FAM, HEX/JOE and Cy5 channels is possible and indicative of a high somatic cell count caused by pathogens different from those detected with this kit.

Table 4. Result interpretation table*

Reporter (pathogen)		Reporter (internal control)		Sample result
FAM	HEX/JOE	Cy5	Texas Red/ ROX	
X			X	Positive for <i>S. aureus</i>
	X		X	Positive for <i>S. agalactiae</i>
		X	X	Positive for <i>M. bovis</i>
X	X		X	Positive for <i>S. aureus</i> and <i>S. agalactiae</i>
X		X	X	Positive for <i>S. aureus</i> and <i>M. bovis</i>
	X	X	X	Positive for <i>S. agalactiae</i> and <i>M. bovis</i>
X	X	X	X	Positive for <i>S. aureus</i> , <i>S. agalactiae</i> and <i>M. bovis</i>
			X	Negative
				Invalid

* Interpretation of sample results can be determined only if positive and negative control reactions are performed. The positive control must yield a signal in all channels (FAM, HEX/JOE, Cy5 and Texas Red/ROX). The negative control must yield no signal in any channel. For a complete explanation of possible sample results, refer to "Data Analysis and Interpretation", page 15.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Please note that in cases of prior bacterial enrichment or bacterial culturing, the internal control may be extremely weak or not amplified at all. In such cases, a missing Texas Red/ROX signal of the internal control does not render the PCR invalid.

Ordering Information

Product	Contents	Cat. no.
<i>bactotype</i> Mastitis HP3 PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	280045
Related products		
DNeasy Mastitis Mini Kit (192)	For 192 preps: QIAamp® Mini Columns, Pathogen Lysis Microtubes S (racked), Buffers, Collection Tubes (2 ml)	69805
MagAttract Mastitis Kit (384)	For 384 preps: Pathogen Lysis Microtubes S (racked), MagAttract Suspension G, Buffers and Reagents	947757
<i>bactotype</i> MAP PCR Kit (96)*	For 96 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285905
<i>virotype</i> ® BTV pan/8 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280445
<i>virotype</i> BTV pan/4 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280455

* Other kit sizes are available; see www.qiagen.com.

virotype BVDV
RT-PCR Kit (96)* For 96 reactions: PCR Mix,
Enzyme Mix, Positive Control,
Negative Control

280375

virotype SBV RT-
PCR Kit (96)* For 96 reactions: Master Mix,
Positive Control, Negative
Control

281605

* Other kit sizes are available; see www.qiagen.com.

QIAGEN offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit **www.qiagen.com/Animal-and-Veterinary-Testing** for more information about *bactotype*, *cadof*[®], *cattletype*[®], *flocktype*[®], *pigtype*[®] and *virotype* products.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Limited License Agreement for *bactotype* Mastitis HP3 PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

The purchase of this product allows the purchaser to use it for amplification and identification of nucleic acid sequences for laboratory use only. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

Trademarks: QIAGEN[®], QIAamp[®], Sample to Insight[®], *bactotype*[®], *cador*[®], *cattletype*[®], DNeasy[®], *flocktype*[®], MagAttract[®], *pigtype*[®], *virotype*[®] (QIAGEN Group); Agilent[®] (Agilent Technologies, Inc.); Bio-Rad[®] (Bio-Rad Laboratories, Inc.); Cy[®] (GE Healthcare); Eppendorf[®] (Eppendorf AG); FAM[™], JOE[™] (Life Technologies Corporation); Mx3005P[®] (Stratagene);. Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

1104990 HB-2305-001 © 2016 QIAGEN, all rights reserved.

Australia • techservice-au@qiagen.com
Austria • techservice-at@qiagen.com
Belgium • techservice-bnl@qiagen.com
Brazil • suportetecnico.brasil@qiagen.com
China • techservice-cn@qiagen.com
Denmark • techservice-nordic@qiagen.com
Finland • techservice-nordic@qiagen.com
France • techservice-fr@qiagen.com
Germany • techservice-de@qiagen.com
Hong Kong • techservice-hk@qiagen.com
India • techservice-india@qiagen.com
Ireland • techservice-uk@qiagen.com
Italy • techservice-it@qiagen.com
Korea (South) • techservice-kr@qiagen.com
Luxembourg • techservice-bnl@qiagen.com
Mexico • techservice-mx@qiagen.com
The Netherlands • techservice-bnl@qiagen.com
Norway • techservice-nordic@qiagen.com
Singapore • techservice-sg@qiagen.com
Sweden • techservice-nordic@qiagen.com
Switzerland • techservice-ch@qiagen.com
UK • techservice-uk@qiagen.com

Ordering www.qiagen.com/contact | Technical Support
support.qiagen.com | Website www.qiagen.com