

Kit contents

bactotype Mastitis Env PCR Kit	(96)
Cat. no.	BT280035
Number of reactions	96
Master Mix (tube with orange cap), includes primers, probes and enzymes	2 x 980 µl
Positive Control (tube with red cap)	1 x 150 µl
Negative Control (tube with blue cap)	1 x 150 µl
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Intended use

The bactotype Mastitis Env PCR Kit is intended for the detection of DNA from *Escherichia coli*, *Klebsiella*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Trueperella pyogenes* in ruminant milk (quarter milk samples, pool or bulk milk).

For veterinary use only.

Bactotype Mastitis Env PCR KIT

Symbols



Legal manufacturer



Lot number



Use by date



Temperature limitations for storage



Handbook



Catalog number



Material number



Protect from light



For ruminant samples

Quality control

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

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Storage

The components of the bactotype Mastitis Env 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 (>2x), 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 from your local sales representative or by Email request under **compliance@indical.com**.

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

Introduction

The bactotype Mastitis Env PCR Kit is a highly sensitive and specific solution for detection of DNA from the most important mastitis-causing environmental pathogens *Escherichia coli* (*E. coli*), *Klebsiella*, *Streptococcus dysgalactiae* (*S. dysgalactiae*), *Streptococcus uberis* (*S. uberis*) and *Trueperella pyogenes* (*T. pyogenes*) in samples from ruminant milk (quarter milk samples, pool or bulk 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 requires 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 Env PCR Kit detects the most important environmental bacteria *E. coli*, *Klebsiella*, *S. dysgalactiae*, *S. uberis* and *T. pyogenes* with high sensitivity.

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 identified using fluorescent dyes. These are usually

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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 Env PCR Kit contains all of the necessary reagents for the simultaneous detection of DNA from *E. coli*, *Klebsiella*, *S. dysgalactiae*, *S. uberis* and *T. pyogenes* including a positive and negative control.

The kit uses four specific primer/probe combinations:

- FAM™ fluorescence for DNA from *E. coli* and *Klebsiella*
- JOE™ fluorescence for DNA from *S. dysgalactiae*
- Texas Red® fluorescence for DNA from *T. pyogenes*
- Cy®5 fluorescence for DNA from *S. uberis*

A Positive Control serves to verify the functionality of the reaction mix for the amplification of one of the DNA targets per channel.

Note: The milk samples can be tested in combination with other *bactotype* Mastitis PCR Kits, which contain an additional primer/probe combination for the detection of an internal control, which is present within the sample.

DNA extraction

The bactotype Mastitis Env PCR Kit can be used for 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. INDICAL recommends using the following products for DNA extraction from milk samples.

Extraction based on magnetic beads:

- MagAttract® Mastitis Kit

Extraction based on spin columns:

- DNeasy® Mastitis Mini Kit

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 viral nucleic acids
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen real-time cycler
- Appropriate strip tubes and caps or 96-well optical microplate with optical sealing film or cover for chosen 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 as assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Negative control

At least one negative control reaction should be included in each PCR run, containing all the components of the reaction except for the pathogen template. 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 µl of the Positive Control provided with the bactotype Mastitis Env PCR Kit to test for successful amplification of the target.

Protocol: Real-time PCR for simultaneous detection of Mastitis-causing pathogens

Important points before starting

- Please read „Important notes“ on 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, spin the reagents briefly.

Procedure

- 1. Pipet 20 µl of the Master Mix into each reaction tube. Then add 5 µl of the sample DNA (Table 1).

Include positive and negative control reactions.

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

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

Table 1. Preparation of reaction mix

Component	Volume
Master Mix	20 µl
Sample	5 µl
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 cyclor according to Table 2.

Note: For Agilent Mx3005P cyclers, use the Factory Defaults Filter Gain Setting (Cy5 1x, ROX 1x, HEX/ JOE 1x, FAM 8x).

Table 2. Filter settings for the reporter

Pathogen/ Internal Control	Reporter
<i>E. coli</i> / <i>Klebsiella</i>	FAM
<i>S. dysgalactiae</i>	JOE/ HEX ¹
<i>T. pyogenes</i>	Texas Red/ ROX ²
<i>S. uberis</i>	Cy5

1 Use the option appropriate for your thermal cycler.

2 Do not use ROX as passive reference or reference dye on ABI 7500 and Agilent Mx3005P.

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

Table 3. Real-time PCR protocol for bactotype Mastitis PCR kits

Step	Temperature	Time	Number of cycles
Initial Activation	95°C	5 min	1
2-step cycling			
Denaturation	95°C	10 s	40
Annealing/Extension*	57°C	30 s	

* Fluorescence data collection. Approximate run time 65 min (Agilent Mx3005P)

Data analysis and interpretation

Interpretation of results

For the assay to be valid:

- The Positive Control yields a signal in all four channels (FAM, JOE/ HEX, Texas Red/ ROX and Cy5) with a $C_T^1 < 35$.
- The Negative Control does not yield a signal in any of the four channels.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 4 on page 17.

Check that there is a fluorescence signal in the pathogen channels (FAM, JOE/ HEX, Texas Red/ ROX, 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.

Note: The milk samples can be tested in combination with other *bactotype* Mastitis PCR Kits, which contain an additional primer/probe combination for the detection of an internal control, which is present within the sample.

¹ C_T , Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence

Table 4. Results interpretation table¹

FAM	JOE/ HEX	Texas Red/ ROX	Cy5	Sample result
Positive for:				
X				<i>E. coli</i> <u>and/</u> or <i>Klebsiella</i>
	X			<i>S. dysgalactiae</i>
		X		<i>T. pyogenes</i>
			X	<i>S. uberis</i>
X	X			<i>E. coli</i> / <i>Klebsiella</i> <u>and</u> <i>S. dysgalactiae</i>
X		X		<i>E. coli</i> / <i>Klebsiella</i> <u>and</u> <i>T. pyogenes</i>
X			X	<i>E. coli</i> / <i>Klebsiella</i> <u>and</u> <i>S. uberis</i>
X	X	X		<i>E. coli</i> / <i>Klebsiella</i> <u>and</u> <i>S. dysgalactiae</i> <u>and</u> <i>T. pyogenes</i>
X	X		X	<i>E. coli</i> / <i>Klebsiella</i> <u>and</u> <i>S. dysgalactiae</i> <u>and</u> <i>S. uberis</i>
X		X	X	<i>E. coli</i> / <i>Klebsiella</i> <u>and</u> <i>T. pyogenes</i> <u>and</u> <i>S. uberis</i>
X	X	X	X	<i>E. coli</i> / <i>Klebsiella</i> <u>and</u> <i>S. dysgalactiae</i> <u>and</u> <i>T. pyogenes</i> <u>and</u> <i>S. uberis</i>
	X	X		<i>S. dysgalactiae</i> <u>and</u> <i>T. pyogenes</i>
	X		X	<i>S. dysgalactiae</i> <u>and</u> <i>S. uberis</i>
	X	X	X	<i>S. dysgalactiae</i> <u>and</u> <i>T. pyogenes</i> <u>and</u> <i>S. uberis</i>
		X	X	<i>T. pyogenes</i> <u>and</u> <i>S. uberis</i>
negative				

¹ Interpretation of sample results can be determined provided positive and negative control reactions are performed. The positive control must yield a signal in all channels (FAM, JOE/ HEX, Texas Red/ ROX and Cy5). The negative control must yield no signal in any channel. For further explanation of the results, please refer to "Data analysis and interpretation" on page 15.

Bactotype Mastitis Env PCR KIT

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, pigtype and virotype products.

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Bactotype Mastitis Env PCR KIT

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