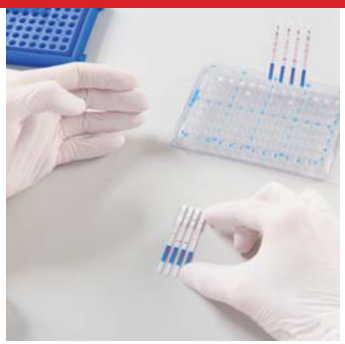
milenia biotec

Lateral Flow Rapid Tests *and more*



Detection of Beer Spoiling Germs

simple, fast, reliable

Milenia GenLine

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About us

Milenia Biotec GmbH was founded in 2000 and is engaged in the development, production and distribution of rapid diagnostic tests based on the technology of lateral flow tests. The best-known example is the Corona rapid test.

The products are very easy to use and can be evaluated either visually or with simple devices. Results are available in a short time.

In order to provide products that meet our customers' expectations in terms of service and quality, Milenia Biotec GmbH has had a quality management system in place since 2003 that is certified by BSI in accordance with DIN EN ISO 13485:2016.

Milenia GenLine is a product line of molecular biological detection systems with a modular structure. All GenLine tests are based on a universal test strip that can detect any molecular biological product. The strips are provided in a PCR Universal Module. The molecular biology modules (PCR Modules) are developed in addition to the strips. This approach allows us to implement new developments quickly and also serve niche markets.

The first products in the Milenia **GenLine product line** are tests for the **detection of beer spoiling bacteria**.

Background

Beer is a medium in which germs find poor growth conditions due to the alcohol content and low pH value. Furthermore, the carbon dioxide and the bitter substances contained in hops represent additional growth barriers for microorganisms.

Nevertheless, some germs have managed to adapt to the beer environment and grow in it. These have beer-damaging potential, as their growth can lead to turbidity and changes in taste.

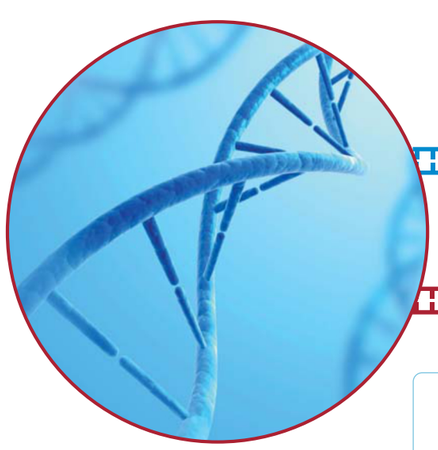
This is why testing for beer spoiling bacteria is an increasing challenge in breweries. The **Milenia GenLine** product line provides a test system for the molecular biological detection of beer spoiling organisms that is **fast, simple and reliable**.

Technology

PCR (polymerase chain reaction) is used as the technological basis for the Milenia GenLine tests.

A DNA polymerase and short single-stranded DNA pieces, called primers, are used to amplify defined DNA sequence regions of a sample in vitro. PCR is based on a temperature protocol consisting of several cycles that are run in succession. In this way, a doubling of the genetic target sequence is achieved per cycle. According to this principle, a single copy of the target DNA segment will result in over 1 billion copies after 30 cycles.

The primers used in the Milenia GenLine tests are provided with specific markers that are recognized by the test strips. The Milenia GenLine test enables the detection of two different gene products on one strip. By adding a control gene to the reaction preparations, a statement can be made as to whether the PCR has been carried out correctly in the specific preparation.



DNA Target Sequence Bacterium

DNA Target Sequence Internal Control

Specific labeled primers

1 Denaturation approx. 95°C

DNA double strands are "melted" at high temperatures into their single strands.

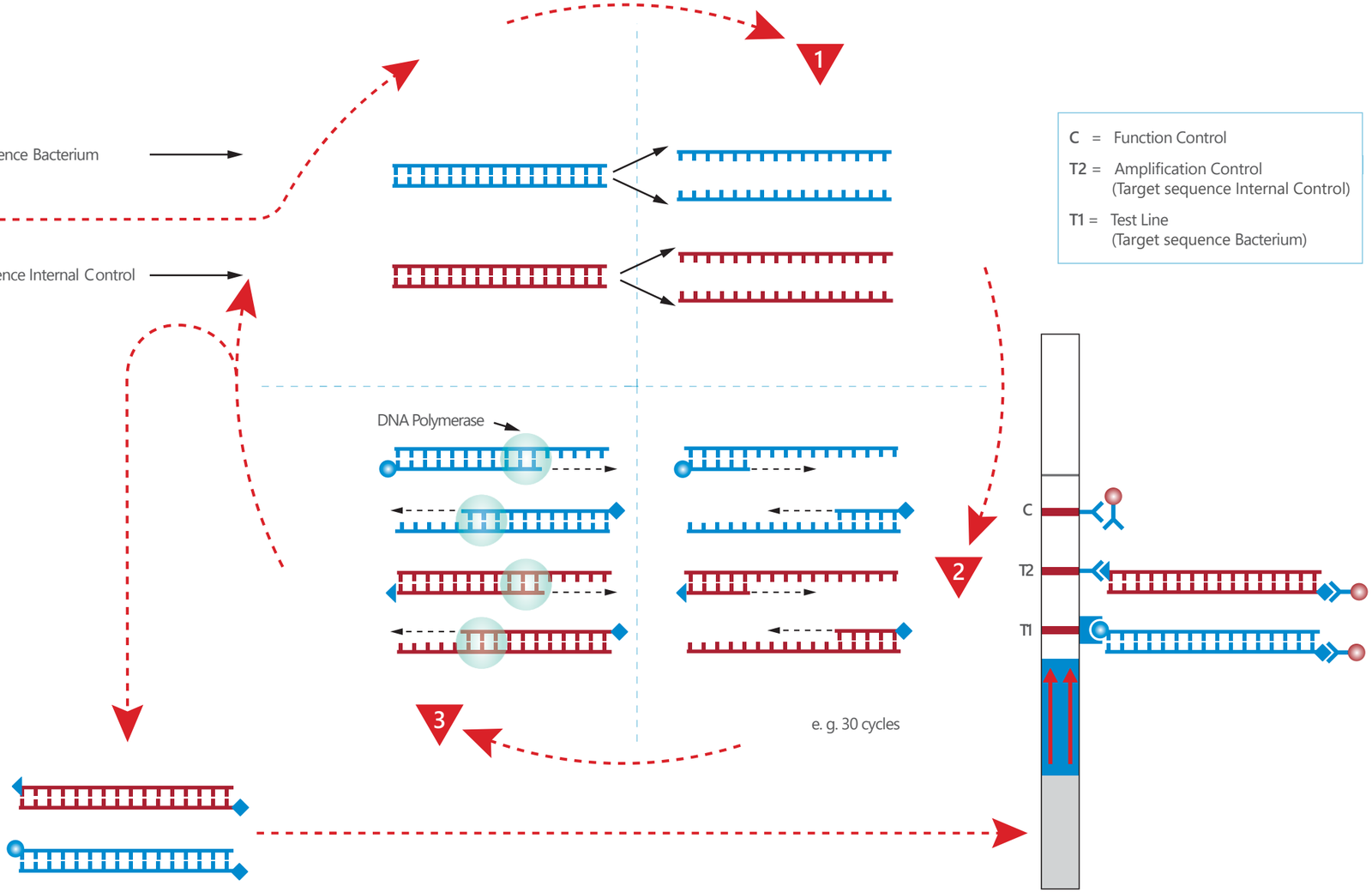
2 Annealing <72°C

Specific labeled primers bind to the single strands..

3 Elongation 72°C

A heat-stable DNA polymerase attaches to the primers and extends the double strands, which serve as starting material for the next cycle.

Technology



C = Function Control

T2 = Amplification Control (Target sequence Internal Control)

T1 = Test Line (Target sequence Bacterium)

Laboratory Equipment Needed

- Simple Thermal Cycler
- Pipettes (2-20µl and 20-200µl)
- Pipette Tips
- PCR Reaction Tubes
- Eppendorf Tubes
- Stand for Reaction Tubes
- Mini Centrifuge
- Vortexer
- Freezer

Sample Preparation

The Milenia GenLine tests for the detection of beer spoiling bacteria can be carried out without DNA isolation from the sample. Most common liquid enrichment media are suitable as sample material. These include the media from Döhler (NBB-B, NBB-B-AM, NBB-C), other MRS-based media, wort and beer.

In-house media can be tested for compatibility very easily.
Yeast-containing samples can also be analyzed directly.

Furthermore, the Milenia GenLine tests allow direct analysis of single colonies of solid media (media-independent).

1. Testing from Liquid Enrichment



2. Direct Analysis of Single Colonies on Solid Media



Test Procedure

1

Pipette **2 µl** of the prepared samples into PCR tubes, place in the thermal cycler and start the program.

2

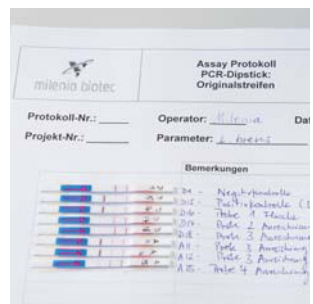
Remove the PCR tube from the thermal cycler and pipette **2 µl** onto the sample application site of the test strip.

3

Place the strip in a container (e.g. microtiter plate) containing **80 µl** of running buffer.

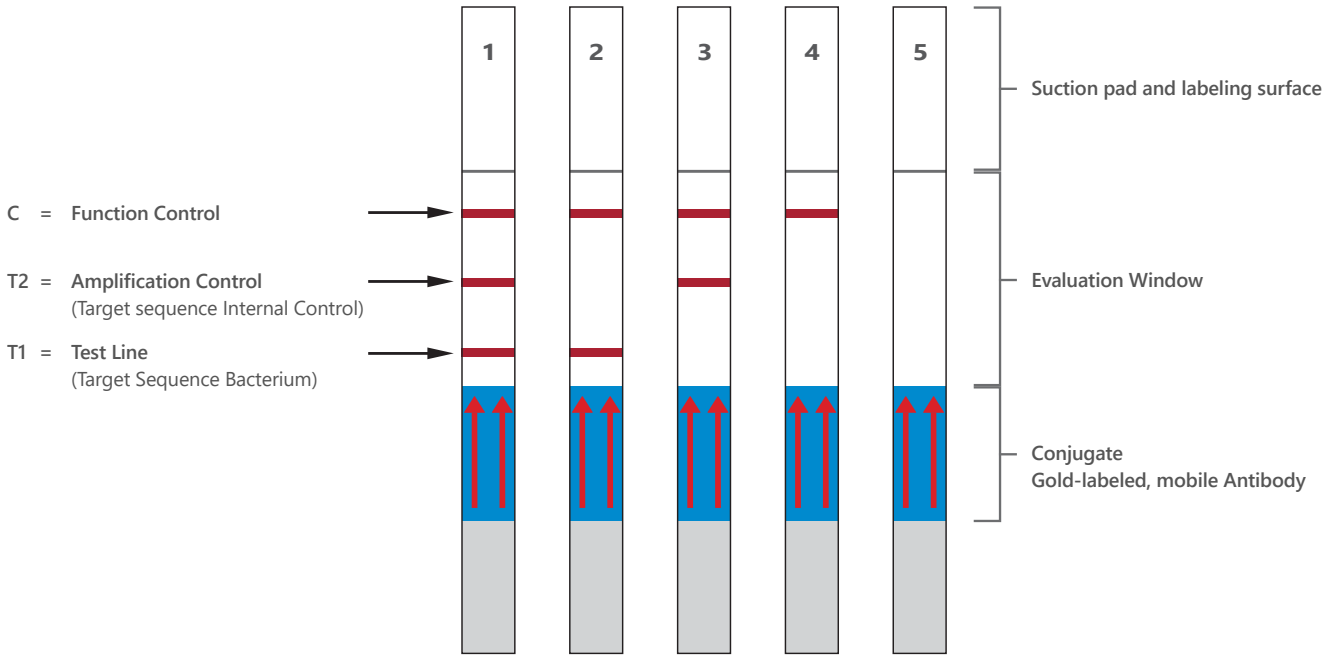
4

Read the test strips after **5 minutes**.



Interpretation of the Test Results

Possible Results of the Evaluation of PCR Products using the Universal Test Strip (REF MGUP 1)



- Strips **1** and **2** are to be interpreted as clearly positive findings.
- Strip **3** corresponds to a negative result.
- Strip 4 indicates a complete inhibition of the PCR.
  This is not a valid negative result, but a sample that cannot be evaluated; the sample must be repeated.
- Strip **5** cannot be evaluated.

↑↑ The red arrows on the test strip indicate the running direction.

Milenia GenLine *Lactobacillus/Pediococcus* Screening

Intended Use

The Milenia GenLine ***Lactobacillus/Pediococcus*** **Screening** is a confirmation and screening test for the most common beer spoilers. The test detects the most important spoilers of the genera *Lactobacillus* and *Pediococcus*, including *Lactobacillus brevis, Lactobacillus lindneri, Lactobacillus casei* and *Pediococcus damnosus*. The Lactobacilli used for biological acidification (e.g. *Lactobacillus delbrueckii*, *Lactobacillus amylovorus*, etc.) and the most important brewery-relevant yeasts are not detected.

Therefore, the Milenia GenLine ***Lactobacillus/Pediococcus*** **Screening** is a particularly useful tool for the assessment of microbiological sample material that is difficult to interpret, for example yeast-containing samples or non-selective enrichments, such as swab samples enriched in NBB-B-AM.

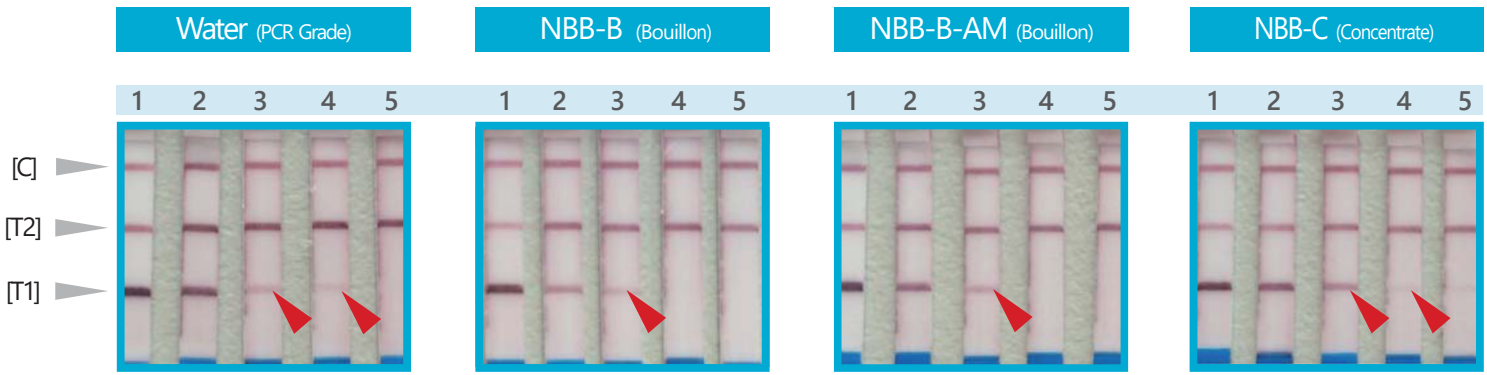
detectable ✓	not detectable x
L. brevis	*L. delbrueckii*
L. backi	*L. amylovorus*
L. casei	*L. amylolyticus*
L. paracasei	*Megasphaera sp.*
L. collinoides	*Pectinatus sp.*
L. paracollinoides	*Acetobacteriaceae*
P. damnosus	*(Enterobacteriaceae)*
L. lindneri	*Torulaspora delbrueckii*
L. plantarum	*Wickerhamomyces anomalus*
L. harbinensis	*Saccharomyces pastorianus 34/70*
L. rossiae	*Saccharomyces cerevisiae 68*
L. coryniformes	*Saccharomycodes ludwigii*
L. acetotolerans	
L. frisingensis	
L. perolens	
P. claussenii	
P. inopinatus	

Milenia GenLine *Lactobacillus/Pediococcus* Screening

Sensitivity / Media used

The Milenia GenLine tests can be used for direct analyses from enrichments. No DNA extraction is required. This considerably reduces the workload and complexity of processing.

All Milenia GenLine detections are compatible with common detection media. Processing by direct analysis works both from liquid enrichments and as colony analysis of solid media.



L. brevis: 1821B	
1	ca. $1x10^6$ cfu/mL
2	ca. $1x10^5$ cfu/mL
3	ca. $1x10^4$ cfu/mL
4	ca. $1x10^3$ cfu/mL
5	ca. $1x10^2$ cfu/mL

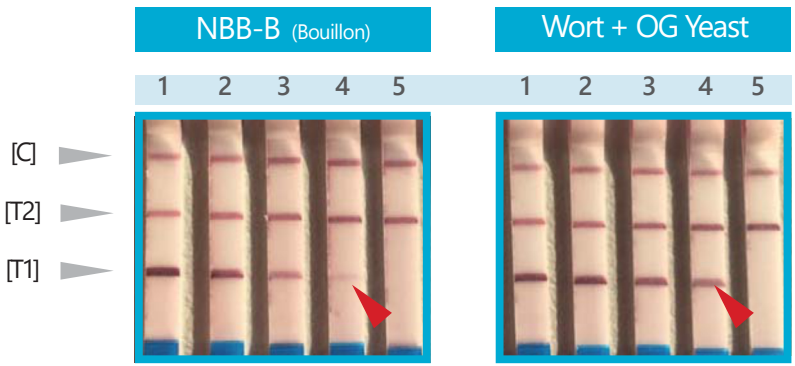
Sensitivity:			
PCR Water	→	~2-20	Cells
NBB-B	→	~20-200	Cells
NBB-B-AM	→	~20-200	Cells
NBB-C	→	~20-200	Cells

Milenia GenLine *Lactobacillus/Pediococcus* Screening

Sensitivity / Yeast containing Samples

The analysis of yeast-containing samples is often particularly difficult in breweries. With the Milenia GenLine tests, it is possible to carry out direct measurements from yeast-containing samples with very good sensitivity.

The detections are therefore a valuable tool for analyzing sample matrices that are difficult to interpret.



L. brevis: 1821B	
1	ca. $1x10^7$ cfu/mL
2	ca. $1x10^6$ cfu/mL
3	ca. $1x10^5$ cfu/mL
4	ca. $1x10^4$ cfu/mL
5	ca. $1x10^3$ cfu/mL

Sensitivity:

→ ~ 20-200 Cells

Performance Data Milenia GenLine *Lactobacillus/Pediococcus*

		Milenia (LB-PC-Screen)		
		+	-	Total
RT-PCR (sLP600, Brandl 2006)	+	16	0	16
	-	3	31	34
Total		19	31	50

Concordance index 0.91

Milenia GenLine Hop Resistance Gene Screening

Background Information

Beer is a difficult habitat for microorganisms to colonize. However, some specialists have managed to tolerate this environment and grow in beer. An often decisive factor for microbial growth in beer is the ability to tolerate hop bitters (primarily iso-alpha-acids). The Milenia GenLine **Hop Resistance Screening** identifies two of the most important cross-species genetic markers for resistance to hop bitterness.

If the bacteria detected have the corresponding genes, it is highly probable that they are strong spoilage organisms with a considerable product damage potential.

Combination and Comparison with the *Lactobacillus/Pediococcus* Screen

The Milenia GenLine **Hop Resistance Screening** is suitable as a confirmation and screening test for obligate beer spoilage members of the genera *Lactobacillus* and *Pediococcus*. This test can be used to assess the harmful potential of potentially beer spoiling bacteria. This is therefore a new type of tool that provides information in less than two hours that previously required more than 14 days using the classic detection method.

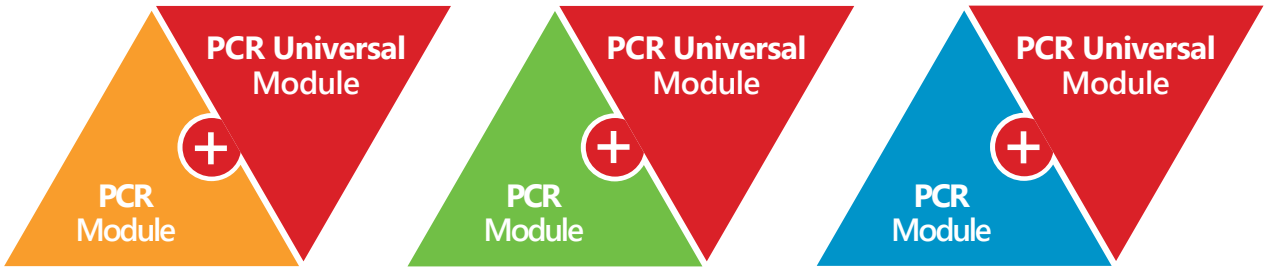
No.	Organism	Milenia GenLineTests		Cultivation						
		Lacto-Pedio-Screen	Hop Resistance Screen	NBB-B-AM (Bouillon)	NBB-B (Bouillon)	Hefeweizen	Lager	Pilsener	Pale Ale	Double IPA
	Alcohol Content					<0.04	5.2	5.1	5.7	8.3
	pH Value					4.36	4.73	4.46	4.79	4.91
	IBU					15	24	32	36	83
-	Negative Control	-	-	-	-	-	-	-	-	-
1	*Lactobacillus brevis I*	+	+	+	+	+	+	+	+	+
2	*Lactobacillus brevis II*	+	+	+	+	+	+	+	-	-
3	*Lactobacillus lindneri I*	+	+	+	+	+	+	+	+	+
4	*Lactobacillus lindneri II*	+	+	+	+	+	+	+	-	+
5	*Lactobacillus backi 2334*	+	+	+	+	+	-	+	+	+
6	*Pediococcus damnosus*	+	+	+	+	+	+	+	+	+
7	*Lactobacillus rossiae I*	+	+	+	+	+	+	-	-	-
8	*Lactobacillus rossiae II*	+	-	+	+	-	-	-	-	-
9	*Lactobacillus casei 610*	+	-	+	+	-	(+)*	(+)*	-	(+)*
10	*Lactobacillus parabuchneri*	+	-	+	+	n.a.	-	-	-	-
11	*Lactobacillus plantarum*	+	-	+	+	-	-	-	-	-
12	*Leuconostoc mesenteriodes*	-	-	+	+	-	-	-	-	-
13	*Lactococcus lactis*	-	-	+	+	n.a.	-	-	-	-

Analyses were carried out in duplicate, * bacterial growth was only recorded in 1/2 cultures; in these samples a renewed test for hop resistance was positive

Performance Data Milenia GenLine Hop Resistance Gene Screening

		Milenia Hop Resistance Gene Screen		
		+	-	Total
RT-PCR (TUM FZWBLQ, Hor A or Hor C Gene)	+	10	0	10
	-	0	7	7
Total		10	7	17
			Concordance index 1	

Available Products



PCR Universal Module	MGUP 1	48 Tests

The Universal Module is always used in combination with the PCR Modules.

PCR Modules

Lactobacillus/Pediococcus Screen	MGScLP 1	48 Tests
Hop Resistance Gene Screen	MGScHOR 1	48 Tests
Megasphaera/Pectinatus Screen	MGScMP 1	48 Tests

Features and Advantages of Milenia GenLine

- Sensitive and Specific
- Internal PCR Control
- Positive Controls included in the Test Kit
- Direct Sample Application without DNA Purification
- Low Costs in Devices
- Low Influence of Yeast
- Results in 60 Minutes

Contact

Any questions, requests, or suggestions? Feel free to contact us at any time.

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