BACTOTYPE® PCR Amplification Kit

Mycoplasma gallisepticum

Labor Diagnostik
Leipzig

Manual

Technology

The product group BACTOTYPE® PCR Amplification Kit comprises optimised systems for the identification of bacterial pathogens via polymerase chain reaction (PCR):

Highest sensitivity and specificity: All components are carefully developed and perfectly optimised

Easy and comfortable work: The reagents are ready-to-use and ready-to-load

Reliable results: The test kit contains an internal amplification control

The primer pair of the test kit is specific for a gene fragment of *Mycoplasma gallisepticum*. The PCR products are analysed on a conventional agarose gel. The ready-to-load reagents allow direct loading of the samples on the gel without addition of gel loading buffer. False negative results are minimized by using an internal amplification control. Manual work is reduced to a minimum. With exception of Taq DNA Polymerase, the test kit consists of all reagents necessary for the detection of the bacterial germs.

For research use only // **08/2006**

Pathogenic Germ

The test kit has been developed for detection of *Mycoplasma gallisepticum* from tracheal swabs.
M. gallisepticum is the pathogen of chronic respiratory diseases (CRD) that leads to chronic diseases of the respiratory tracs in chicken including trachitis, pneumonia and/or serositis. As multifactorial-induced infection CRD is associated frequently with Newcastle Diseases, infective bronchitis or laryngotrachitis. Infected turkey hens tend to show primarily sinusitis. *M. gallisepticum* is passed aerogenly or via eggs. BACTOTYPE® PCR Amplification Kit *Mycoplasma gallisepticum* substitutes either serological diagnosis and cultivation of bacteria and provides sensitive and specific results in about one day.

Ordering Information

BACTOTYPE® *Mycoplasma gallisepticum*	10 Reactions	Cat. No. 04-107/10
BACTOTYPE® *Mycoplasma gallisepticum*	50 Reactions	Cat. No. 04-107/50

Content

	10 reactions	50 reactions
PCR Mix (yellow cap) PCR buffer, dNTPs, primer mix (species-specific and amplification control), DNA for amplification control, NaN_3	221 µL	935 µL
Magnesium Chloride (green cap) $MgCl_2$, gel loading buffer	80 µL	300 µL
Positive Control (red cap) non-infectious DNA with germ-specific sequences, NaN_3	25 µL	25 µL
Nuclease-free Water (blue cap)	1.0 mL	1.0 mL

Storage Conditions

Reagents of the test kit are stable for at least 12 months when stored at -20 °C in darkness. Repeated freezing and thawing should be avoided.

Additionally Reagents

- Taq DNA Polymerase
  BACTOTYPE® test kits are validated with JumpStart™ Taq DNA Polymerase (Sigma-Aldrich, Cat. No. D4184). Solely, the use of this DNA Polymerase assures precise function of the BACTOTYPE® products. For ordering and further information, please contact Sigma-Aldrich.
- Agarose and Ethidium bromide (for gel electrophoresis)
- 100 bp DNA ladder (for gel electrophoresis)

Trademarks and Patents

- BACTOTYPE® is a registered trade mark of the Biotype AG.
- JumpStart™ is a registered trademark of Sigma-Aldrich.
- The PCR is under patent laws. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).

Product Warranty

All contents of the BACTOTYPE® PCR Amplification Kit are subjected to extensive quality procedures by Labor Diagnostik Leipzig. In case of any problem, please report immediately.

Notes

Please, carry out precisely the instructions of the protocol!

In order to avoid any kind of contamination we recommend pipetting with filter tips. DNA preparation, amplification, and electrophoresis should be carried out in separate rooms. Take note of safety regulations for working in laboratories, and wear gloves.

The PCR Mix and the Positive Control contain sodium azide (NaN_3). Sodium azide is hazardous: Very toxic if swallowed, develops toxic gases when it gets in contact with acids (R28-32-50/53, S1/2-28-45-60-61). Ethidium bromide is hazardous, too. Please refer to the Material Safety Data Sheet (MSDS) and wear nitrile gloves.

MSDS of all BACTOTYPE® components are available from Labor Diagnostic Leipzig. For MSDS of additional reagents to be needed, please contact the corresponding manufactures.

DNA Isolation

Solely, ultrapure DNA implies the successful amplification. For DNA isolation of *Mycoplasma gallisepticum* from tracheal swabs the following test systems are recommended:

- NucleoSpin® Tissue Kit; Macherey-Nagel, Düren
- QIAamp® DNA Mini Kit; QIAGEN, Hilden

Please perform isolation according to the manufacturer's information.

Protocol

Preparation of the reagents: Before using the reagents of the test kit the first time, mix thoroughly and centrifuge the reagents for 5 sec at 3000 rpm. The blue colour in the magnesium chloride solution arises from the gel loading buffer and does not affect the PCR reaction. Please, avoid elongated centrifugation steps of the magnesium chloride solution. The gel loading buffer could be concentrated onto the bottom of the tube.

1. A) PCR Protocol for a Number of Samples different from the Packaging Unit (Multiplicator)

If there are less samples than the packaging unit, pipette as followed into a PCR reaction tube (also see pipetting scheme table 1):

17.0 µL PCR Mix
+ 4.5 µL Magnesium Chloride
+ 0.6 µL Nuclease-free Water
+ 0.4 µL Taq DNA Polymerase (here: JumpStart™ 2.5 U/µL)

- Pipet 22.5 µL of the master mix into a PCR reaction tube and add 2.5 µL of your DNA sample or control.
- **Attention:** Depending on the used Taq Polymerase, complete with Nuclease-free Water to a total volume of 25 µL per reaction.

1. B) PCR protocol for an Amount of Samples corresponding to Packaging Unit

If the number of samples corresponds to the packaging unit, we recommend the addition of Magnesium Chloride, Nuclease-free Water, and Taq DNA Polymerase directly to the PCR Mix.

For 10 samples (corresponding to the content of 10 reactions)

PCR Mix
+ 58.5 µL Magnesium Chloride
+ 7.8 µL Nuclease-free Water
+ 5.2 µL Taq DNA Polymerase (here: JumpStart™ 2.5 U/µL)

For 50 samples (corresponding to the content of 50 reactions)

PCR Mix
+ 247.5 µL Magnesium Chloride
+ 33 µL Nuclease-free Water
+ 22 µL Taq DNA Polymerase (here: JumpStart™ 2.5 U/µL)

- Pipet 22.5 µL of the master mix into a PCR reaction tube and add 2.5 µL of your DNA sample or control.

2. Positive Control

The test kit is provided with a ready-to-use Positive Control (red cap) of non-infectious DNA with germ-specific sequences. We strongly recommend performing a positive control for each run.

3. Negative Control

It is strongly recommended to perform a negative control (e. g. Nuclease-free Water) for each run.

Table 1: Pipetting Scheme

	Sample (x1)	Positive Control	PCR Control
PCR Mix	17.0 µL	17.0 µL	17.0 µL
Magnesium Chloride	4.5 µL	4.5 µL	4.5 µL
Sample DNA	2.5 µL	-	-
Positive Control	-	2.5 µL	-
Nuclease-free Water	0.6 µL	0.6 µL	3.1 µL
Taq DNA Polymerase (1 U)*	0.4 µL	0.4 µL	0.4 µL

* Use 1 Unit for amplification (here: JumpStart™ 2.5 U/µL).

The volume of the DNA to be employed depends on its concentration and may be varied from 1 to 3 µL. Adjust the volume with Nuclease-free Water to a reaction volume of 25 µL in total.

PCR Amplification Parameters

We strongly recommend the use of Sigma JumpStart™ Taq DNA Polymerase.

Temperature	**Time**	
94°C	3 min	Hot Start*
94°C	30 s	
60°C RAMPING 1°C/s**	30 s	35 Cycles
72°C	30 s	
72°C	5 min	
10°C	∞	hold

* For "hot start" Polymerase, please follow the activation time conditions specified by the manufacturer (for JumpStart™ Taq DNA Polymerase: 3 minutes).

** The value is adjusted for most of the thermocyclers. Cyclers that exceed the ramping rate should be monitored and corrected as required.

4. Electrophoresis

PCR fragments are analysed on a 2% agarose gel and staining is done with ethidium bromide (0.5-1.0 µg/mL) in TAE buffer.

Put 10 µL of the PCR product per lane onto the gel (gel loading buffer already included). In order to compare the molecular size of the amplification products a size standard (100 bp DNA ladder) is recommended.
Run the electrophoresis as described in the manufacturer's handbook.

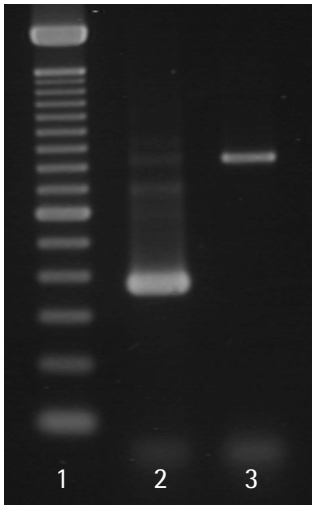
5. Analysis and Interpretation of the Results

Size designation of the PCR fragments is done by comparison with the DNA size standard on a transilluminator. Photographic documentation is recommended.
Protect eyes and skin against UV-radiation!

Positive result: The germ-specific amplificat of 376 bp (base pairs) is clearly visible. The 838 bp (amplification control) band may be weak or may not appear at all due to low concentrations of bacterial DNA.

Negative result: If the 838 bp band (amplification control) appears only, germ detection is considered to be negative.

Invalid result: If none of the bands mentioned above are detectable, the assay is invalid and has to be repeated. Possibly, the PCR has been inhibited.



838 bp amplification control

376 bp germ-specific band

Lane 1: 100 bp DNA ladder (Invitrogen)
Lane 2: germ-specific signal (376 bp); sample is positive
Lane 3: only the amplification control is visible (838 bp); sample is negative

Please contact us for further information.