Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

XpressMyco qPCR Mycoplasma Detection Kit

Catalog No.: XMYCQ-25, XMYCQ-100, XMYCQ-250

Description

The XpressMyco qPCR Mycoplasma Detection Kit is designed for the direct detection of *Mollicutes,* such as *Mycoplasma, Acholeplasma,* and *Sprioplasma* in cell culture media and other biological matrices. Each kit contains all the necessary qPCR components including hot-start Taq polymerases, primers, and dNTPs. The Master Mix includes internal control DNA to reliably identify PCR inhibition and/or DNA extraction issues.

Contents

Quantity				
Component	**25 Reactions**	**100 Reactions**	**250 reactions**	**Cap Color**
Master Mix (lyophilized)	1 vial	4 vials	10 vials	Red
Rehydration Buffer	1 x 1.8 ml	2 x 1.8 ml	5 x 1.8 ml	Blue
Positive Control DNA (lyophilized)	1 vial	1 vial	1 vial	Green
PCR Grade Water	1 x 2.0 ml	1 x 2.0 ml	1 x 2.0 ml	White

Storage: 2 - 8°C, rehydrated mix must be stored at -20°C

Additional Materials Needed

- qPCR device with FAM and HEX filters
- PCR reaction tubes for corresponding qPCR device
- 1.5 ml reaction tubes, DNase – and RNase – free
- Microcentrifuge for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)
- Optional for carry-over prevention: Uracil DNA glycosylase (UNG)

Precautions

The XpressMyco qPCR Mycoplasma Detection Kit is for research use only. Read entire kit manual prior to the procedure. All samples should be considered as potentially infectious and handled accordingly while wearing appropriate PPE. The kit does not contain any hazardous substances, remnants can be discarded according to local regulations.

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Notes

- Use entire kit as a unit, do not mix reagents supplied from multiple kit lots.
- Set up at least one negative control sample (non-template control) in each PCR. Use the elution buffer for the NTC in case of extracted DNA.
- The control samples must be processed in the same manner as the test samples. Other laboratory specific control samples such as high, medium, and low DNA levels (e.g. 3 x LOD_{95}) may be included.

Sample Preparation

Samples should be collected when cell cultures reach 80 to 90% confluence. Cell culture supernatants do not require additional sample preparation. The average mycoplasma concentration in cell culture is ~10^6 particles per ml, with a maximum of 10^8 particles per ml. Within this range, enough mycoplasma DNA is present in the supernatant for successful application of the qPCR test.

PCR inhibiting substances may accumulate in cell culture medium, which will make it necessary to extract the DNA prior to the PCR test (*see below for additional information). Note: penicillin or streptomycin in cell culture media are not known to inhibit mycoplasma or affect the test's sensitivity.

Prepare qPCR template as follows:

1. Transfer 100 µl of cell culture supernatant to a sterile 1.5 ml reaction tube.
2. Incubate sample at 95°C for 10 minutes (5 minute minimum).
3. Centrifuge the sample for 30 seconds at max speed (e.g. 10,000 x g) to pellet cellular debris.
4. Use 2 µl of the supernatant directly for qPCR or store the sample for up to 6 days at +2 - 8°C. Store at -20°C for long-term storage.

* Cell pellets cannot be used directly for the test due to the negative influence of cell debris on the PCR reaction. Cell pellets, higher PCR input volumes (>2 µl), or other biological materials such as fetal calf serum (FCS, > 5 %), vaccines, cryo stocks, and paraffin-embedded samples require DNA extraction prior to PCR. Extracted DNA can be stored at +2 - 8°C for up to 6 days or at -20°C for long term storage.

Procedure Note

The test should be carried out with negative and positive controls and samples in duplicates. All reagents and samples must be equilibrated to +2 - 8°C prior to use. After reconstitution, the reagents must be stored at -20°C. Repeated freezing and thawing should be avoided. For small sample numbers, it is recommended to prepare and aliquot reconstituted Master Mix and Positive Control DNA.

Reagent Preparation

1. Centrifuge lyophilized Master Mix (red cap) and lyophilized Positive Control DNA (green cap) at maximum speed for 5 minutes.
2. Add 600 µl Rehydration Buffer (blue cap) to the Master Mix (red cap).
3. Add 300 µl PCR Grade Water (white cap) to Positive Control DNA (green cap).

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4. Incubate reconstituted Master Mix and Positive Control DNA at room temperature for 10 minutes.
5. Vortex both reagents briefly, then centrifuge for 5 seconds.

Procedure

Prepare PCR Reaction:

1. Pipette 23 µl of the Master Mix into each 1.5 ml PCR reaction tube.
2. Add 2 µl of negative control, sample, or Positive Control DNA (green cap) to corresponding reaction tube.

   Note: For negative control, use fresh cell culture medium or elution buffer from DNA extraction kit.
3. Be sure to close reaction tubes tightly, then spin down tubes briefly.

qPCR Amplification:

1. Place the prepared PCR reaction tubes in the qPCR device and close the lid.
2. Program the qPCR cycler.

   Note: For detailed cycler programs, please contact our technical support team at xpressbio@xpressbio.com.
3. Start program to perform qPCR.

Results

The presence of mycoplasma is indicated by an increasing fluorescence signal in the FAM channel. The quantification is based on threshold cycle (C_t) values and a DNA standard curve. The exact procedure for obtaining C_t values including baseline calculation/normalization depends on the particular qPCR device and cycler control software. Please see the documentation of your device for further details. We recommend the assessment of the amplification curve progression of all samples including control samples.

A positive PCR is indicated by $C_t < 40$. PCR reactions with $C_t \geq 40$ are considered negative. In addition, a successful PCR is displayed by an increasing fluorescence signal in either the FAM or the HEX channel, or both. Thus, the more mycoplasma DNA is in the sample, the higher the signal in the FAM channel and the lower the internal control signal in the HEX channel. The following table shows interpretation of PCR results:

Mycoplasma (FAM Channel)	Internal Control (HEX Channel)	Interpretation
positive	irrelevant	positive mycoplasma detection
negative	negative	PCR inhibition
negative	positive	negative mycoplasma detection

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Assay Characteristics

The XpressMyco qPCR Detection Kit is designed with the included primer set to specifically target the 16S rRNA coding region of the mycoplasma genome but does not amplify Eukaryotic DNA. This allows for detection of the mollicute strains in the table listed below:

Acholeplasma laidlawii	Mycoplasma testudinis
Mesomycoplasma conjuctivae	Mycoplasmoides alvi
Mesomycoplasma flocculare	Mycoplasmoides gallisepticum
Mesomycoplasma hypopneumoniae	Mycoplasmoides genitalium
Mesomycoplasma hyorhinis	Mycoplasmoides pneumoniae
Mesomycoplasma ovipneumoniae	Mycoplasmopsis agalactiae
Metamycoplasma arthritidis	Mycoplasmopsis alligatoris
Metamycoplasma hominis	Mycoplasmopsis anatis
Metamycoplasma hyosynoviae	Mycoplasmopsis arginini
Metamycoplasma orale	Mycoplasmopsis bovis
Mycoplasma amphoriforme	Mycoplasmopsis canis
Mycoplasma corcodyli	Mycoplasmopsis columbina
Mycoplasma iguanae	Mycoplasmopsis fermentans
Mycoplama imitans	Mycoplasmopsis hyopharyngis
Mycoplasma mobile	Mycoplasmopsis pulmonis
Mycoplasma testudineum	Mycoplasmopsis synoviae