Xpert Mycoplasma PCR Detection Kit

#GTC30.0100 (100 rxn)
(FOR RESEARCH ONLY)

Product: Contamination of cell cultures and bioprocessing fluids by mycoplasma and acholeplasma species continues to be a major problem. Most often, the species found in cell cultures are from human origin (from laboratory staff). Contamination happens either directly or indirectly (cross-contamination by other infected cell cultures in the same environment). The most common species found in infected cultures are Mycoplasma *orale*, Mycoplasma *fermentans* and Mycoplasma *hominis*, however, many other species and strains have isolated from infected cell cultures.

Undetected mycoplasma contamination can have significant negative impact on cell culture studies as it may affect proliferation, metabolism, gene synthesis and adhesion properties. Therefore, it is very important to detect, and possibly eliminate, any infection. This Xpert Mycoplasma PCR Detection Kit provides a fast, sensitive and reliable method for the detection of Mycoplasma and Acholeplasma species based on PCR using precisely designed specific primers. Over 200 different species of Mycoplasma and Acholeplasma can be detected, including M. *orale*, M. *fermentans*, M. *hominis*, M. *arginini*, M. *hyorhinis*, M. *pneumoniae* and A. *laidlawii* *). The whole procedure can be carried out within 90-120 minutes, without the need of DNA isolation and with a sensitivity of as little as 10 cfu/ml.

*) a comprenhensive list of detectable strains can be found on the product page at our website.

An internal control (IC) is present in the Xpert Myco Mix, which results in a 180bp PCR product that should be present in all samples in order to minimize the risk of false negatives. Moreover, the kit contains a positive control, which should result in a product of approximately 500bp. After PCR, samples can be loaded directly onto an agarose gel without the need of adding a loading dye. Positive samples result in PCR products in the range of 300bp-600bp, depending of the species and strain.

In case samples are found to be Mycoplasma/Acholeplasma-positive, we recommend to treat the cell culture with GRiSP´s Xpert Mycoplasma Elimination Reagent (#GTC31.0001).

Applications: Detection of Mycoplasma contamination in Cell Culture

Contents: The Xpert Mycoplasma PCR Detection Kit contains sufficient reagents for 100 PCR reactions.

Component	GTC30.0100
Xpert Myco Mix A	1.25 ml
Xpert Myco Mix B	100 µl
Positive Control (Myco+)	250 µl
PCR grade water	1.0 ml

Properties: Fast, Easy, and Reliable
Low limit of Detection
No need for DNA isolation

Storage: Store all components at -20ºC.

Prior to use:

Before proceeding with mycoplasma detection, cells should remain 2-3 days undisturbed in culture and be at least 80% confluent.

Protocol

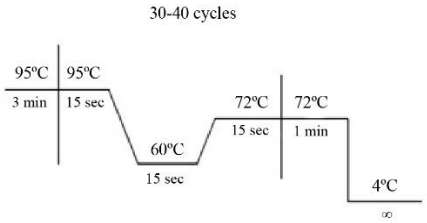
1. Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Xpert Fast:

Component	Volume (25µl)
Xpert Myco Mix A	12.5 µl
Xpert Myco Mix B	1.0 µl
PCR–grade water	9 µl
Sample*) **or** Positive Control **or** NTC	2.5 µl

*) Collect 2.5 µl of the culture media and use directly as sample in the PCR Mix. GRiSP recommends to always include a positive and negative control. For this, use 2.5µl of the included Myco+ as positive control and use 2.5µl of the included PCR-grade water as Negative Template Control (NTC).

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control, and a positive control, by mixing all components (N+2), except template DNA (nor control), dividing the mixture equally into each PCR tube (22.5 µl each), briefly spin tubes (or tap down) and then add 2.5 µl template DNA or control directly in the mixture.

2. Set-up qPCR cycling:



After an initial cycle of 3 min at 95°C (enzyme activation and denaturation of template), cycle 30-40 times for 15 seconds at 95°C, 15 seconds at 60°C and 15 seconds at 72°C, followed by a final extension step at 72°C for 1 minute. Maintain the reaction mixture at +4°C or store at -20°C.

3. DNA Agarose gel electrophoresis:
   Analyze PCR products on a 2% agarose gel. Samples can be loaded directly onto the gel as Mix A contains loading dye.

Results

In order to validate the assay, controls must have the following results. If the signal of one of the controls does not match, the whole experiment, including all samples, must be repeated.

Control	IC (180bp)	300-600bp
NTC	positive	negative
Positive Control (Myco+)	positive	positive (~500bp)

For each sample, there are 4 possible outcomes, as summarized in the table below.

IC (180bp)	300-600bp	Result
positive	negative	negative
positive	positive	positive
negative	negative	inhibition*
negative	positive	positive**

*) in case both Internal Control and target are negative, but all the controls resulted in signals as expected, the sample must be retested, as the PCR reaction was inhibited. Inhibition often is the result of a too high DNA concentration and therefore it is recommended that retesting should be carried out with a 10-fold dilution (using PCR-grade water) of the original DNA sample.
**) in case IC is unclear or even negative but there is a clear positive target band (not a faint band from carry-over from another well), the sample is positive and there is no need to retest.
Note: due to the primer sequences and cycling conditions, it is not uncommon to see a relatively strong primer-dimer band (<100bp) on the gel.