MycoQuick
Mycoplasma Detection Kit

User Manual

Store Detection plate at 4°C
Store lysis buffer at -20˚C

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Quick Reference:

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I. Introduction and Background

A. Mycoplasma Contamination

Mycoplasmas are small, round or filamentous prokaryotic organisms which are a frequent contaminant of cell cultures. It has been estimated that 15%~35% of cells cultures are contaminated with mycoplasma. The contamination can modify many aspects of cell physiology and result in unreliable experimental results. Due to their small size and deformability, mycoplasma can pass or be forced through 0.22 µm filters. The lack of a cell wall makes mycoplasma unresponsive to common antibiotics. It is essential that all cell stocks and new cultures entering a facility are tested for the presence of mycoplasma and all cell cultures should be tested routinely (e.g. once every 2~3 months) in order to maintain a mycoplasma-free environment.

B. Mycoplasma Detection Methods

Many methods are available for detection of mycoplasma, including isolation in broth/agar culture, direct or indirect fluorescence staining, ELISA, immunostaining, direct or indirect PCR. Among those methods, direct PCR is the highly sensitive, specific and convenient method when the primer design is optimized (Ref.).

C. MycoQuick Mycoplasma Detection Kit

The SBI MycoQuick mycoplasma detection kit is capable of detecting mycoplasma infection in cell cultures in less than three hours. It can detect mycoplasma from both cell lysates and cell culture media. The sensitivity is up to 10~20 copies of target DNA, which translates to less than 10 mycoplasma.

The MycoQuick mycoplasma detection kit detects the top five common species of mycoplasma, *M. Hyorhinis, M. Arginini, M. Orale, M. Fermentans, A. laidlawii*, which represent 98% of tissue culture infections. It can also detect *Mycoplasma, Acholeplasma, Ureaplasma, Spiroplasma* from other genera.

A positive control is included to control the successful amplification of PCR reaction and confirm the size of the PCR products in the testing samples. The mycoplasma primers will generate a 280-bp product. The UCE primers will generate a 205-bp band. The UCE primers confirm the absence of PCR inhibitors in all PCR samples. The UCE primers have been tested to detect an ultra-conserved element in human, mouse and rat genome. The amplification of UCE indicates a successful sample preparation and subsequent PCR amplification, which excludes false negative results.

Both mycoplasma primers and UCE primers are coated at the bottom of the each well of 96-well plate. Two amplifications can be achieved simultaneously in one PCR reaction. The two PCR products can be easily separated from each other on a 2.5% agarose gel.

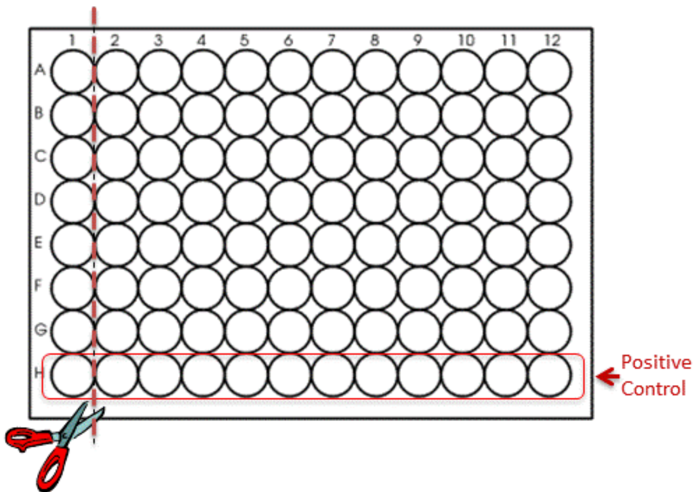
The primers have been adapted for using a variety of PCR enzymes, such as Taq, Pfu, Phusion and SYBR Green mix.

D. Precautionary Notes

1. To prevent contamination of mycoplasma DNA during experimental procedure, always wear gloves during sample preparation and PCR reaction setup.
2. To avoid false positives, water used in PCR reactions can be UV-irradiated.
3. To avoid cross-contamination between samples, aerosol-resistant pipet tips should be used throughout the protocol.

E. Kit Components

1. MycoQuick 96-well plate with mycoplasma primers and UCE primers coated at the bottom of each well. The 12 wells of the last lane also contain positive control DNA templates for mycoplasma and UCE. The plate can be cut into strips according to the number of the samples to be tested.



2. 8-cap strips (×12)
3. Cell lysis buffer (10 ml)

F. Additional Materials and Instruments Needed

1. Table-top centrifuge
2. PCR enzyme and buffer
3. PCR machine/qPCR machine
4. Materials and apparatus for DNA electrophoresis

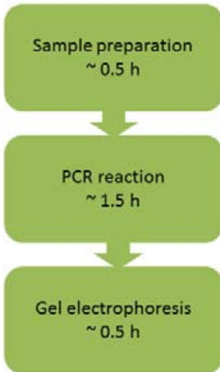
G. Storage

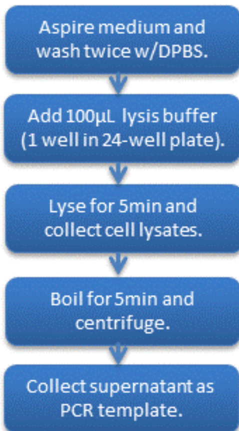
Store 96-well MycoQuick mycoplasma detection plate at 4°C until the expiration date.

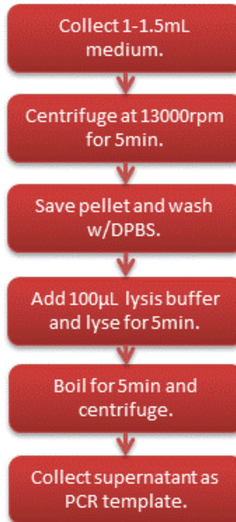
Store cell lysis buffer at 4°C or -20°C until the expiration date.

II. Protocols

A. Flowchart







B. Experimental Procedure

Cells cultured in the absence of antibiotics for several days can enhance the strength of PCR signal. Media sample should be derived from cells that are at least 80% confluent.

Sample Preparation from Cell Lysate

1. Aspire medium from attached cells.
2. Wash cells twice with DPBS.
3. Add appropriate amount of lysis buffer (100 µL per well of 24-well plate). Lyse at room temperature for 5 min and collect cell lysate to an eppendorf tube.
4. Boil lysates at 95 °C for 5 min.
5. Centrifuge the lysates at 13000 rpm for 5 min and collect supernatant.
6. Take 1~2 µL supernatant as template for PCR reaction.

Sample Preparation from Media

1. Collect 1~1.5 mL cell culture medium to an eppendorf tube.
2. Centrifuge at 13000 rpm for 5 min.
3. Discard supernatant and wash the pellet once with DPBS.
4. Add 100 µL lysis buffer to the pellet, pippet up and down, and lyse at room temperature for 5 min.
5. Boil lysates at 95 °C for 5 min.
6. Centrifuge the lysates at 13000 rpm for 5 min and collect supernatant.
7. Take 1~2 µL supernatant as template for PCR reaction

PCR Reaction

MycoQuick 96-well mycoplasma detection plate has been tested for various PCR enzymes, including Taq, Pfu, Phusion and SYBR Green mix …

PCR setup may vary according to the enzyme used. A typical setup with Taq is shown below. A final volume of 25 µL is recommended for each reaction.

Component	Sample reaction /25 µL	+ control reaction /25 µL	- control reaction /25 µL
10×Buffer	2.5	2.5	2.5
dNTP mix (10 mM)	1	1	1
Sample	1	-	-
Taq polymerase	0.2	0.2	0.2
H2O	20.3	21.3	21.3

The general order for setting up PCR is to make the PCR master mix first, add the appropriate amount of mix (e.g. 24 µL) to each well of 96-well plate, and then add 1 µL of cell lysate/medium sample to each well.
Close the wells tightly with the cap strips (provided) and put them into PCR machine.

A typical PCR program with Taq is shown below:

Cycle(s)	Temperature	Time
1	95 °C	30 s
40	95 °C	30 s
	56 °C	30 s
	68 °C	30 s
1	68 °C	5 min

Electrophoresis of PCR Products

For optimal separation between the mycoplasma band and the UCE band, we recommend 2.5% agarose gel for electrophoresis.

1. Mix the final products of each PCR reaction with gel electrophoresis loading buffer.
2. Load each sample together with DNA size marker into individual well of 2.5% agarose gel.

3. Electrophorese at the conditions recommended by the gel box manufacturer.
4. Visualize the bands with ethidium bromide.

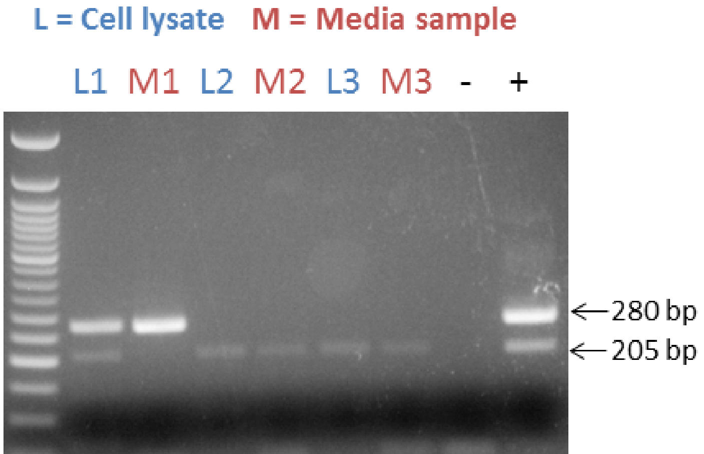
C. Expected Results:

To determine whether the sample is contaminated with mycoplasma, insure that both positive control and negative control give expected results.

PCR template	PCR product(s)	Interpretation
Positive control	280 bp and 205 bp bands	Expected control result
	One/No band	Failed PCR reaction
Negative control	No band	Expected result
	One/Two bands	Contaminated reagents
Sample	280 bp and 205 bp bands	Mycoplasma contamination
	280 bp band only	Heavy mycoplasma contamination
	205 bp band only	No mycoplasma contamination
	No band	Inhibited PCR reaction

If the cell culture is contaminated with mycoplasma, a 280 bp band will be observed on the gel. In addition, a 205 bp band should be amplified from the UCE of the genomic DNA of the hosting cells. If the cell culture is heavily infected with mycoplasma, the amplification of the 280 bp band may result in diminished amplification of the 205 bp internal control. Media samples give a weaker UCE internal control signal than the cell lysate samples.

An example of experimental result is shown below.



Both cell lysate and medium from sample 1 give positive result for mycoplasma with both 280 bp and 205 bp bands or 280 bp band only. Sample 2 and 3 give negative result with 205 bp band only.

III. References

Detection of Mycoplasma in cell cultures. Young L, Sung J, Stacey G, Masters JR. Nat Protoc. 2010;5(5):929-34

IV. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

Phone: (650) 968-2200
(888) 266-5066 (Toll Free)
Fax: (650) 968-2277
E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

System Biosciences (SBI)
265 North Whisman Road
Mountain View, CA 94043

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The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

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