

Mycoplasma Testing Report

Name of the test: Testing of mycoplasma contamination by qPCR method

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Testing of mycoplasma by qPCR method

Experiment scheme

1. Summary:

Use qPCR method to test if sample is contaminated by mycoplasma.

2. Principle of experiment

Use magnetic microbeads to absorb residual DNA in samples then extracted by magnetic separator. Qualitative analysis of starting template is achieved by real-time testing of fluorescence signals corresponding to products from every cycle of amplification.

3. Design of experiment:

3.1 Extract DNA from control samples.

3.2 Set no template control (NC) and negative quality control (NEC) groups to ensure that experiments are not contaminated.

3.3 Test samples with quantitative fluorescence PCR.

Scope of application

Scope: Applicable for experiments of testing mycoplasma contaminations in samples of M00529.

Preparation

1. Instruments and equipment

Instruments and equipment	Manufactures	Types	Number of fixed assets
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qPCR	Thermo Fisher	7500	GR17010005
Centrifuge	Thermo Fisher	FRESCO 17	GR14010111
Dry Thermostat	Ruicheng Instruments Co.,Ltd	DH100-4	CQ18010232
Vortexing instrument	Kyli-Bell Lab Instruments	VORTEX-5	NA

2. Consumables

Names	Manufactures	Cat.No	Lot. No.
Magnetic Particles	Invitrogen	12321D	4217
Filter tips	AXYGEN	TF-400-R-S	P1344535
Filter tips	AXYGEN	TF-20-R-S	23817434
Filter tips	AXYGEN	TF-200-R-S	35717308
Filter tips	AXYGEN	TF-1000-L-R-S	34817001
96 well plates	AXYGEN	PCR-96-AB-C	13717020
1.5ml EP tubes	Eppendorf	022431021	G174706P
2.0ml EP tubes	Eppendorf	022431048	G173343K

3. Reagents

Names	Cat. No.	Lot. No.	Manufactures
PreSEQ™ Nucleic Acid Extraction Kit (Box 1 of 3)	4400793	1801137	Thermo Fisher
PreSEQ™ Nucleic Acid Extraction Kit (Box 2 of 3)	4400795	1712145	Thermo Fisher
PreSEQ™ Nucleic Acid Extraction Kit (Box 3 of 3)	4400675	1802118	Thermo Fisher

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MycoSeq™ Discriminatory Positive/ Extraction Control	4445000	1802063	Thermo Fisher
Mycoplasma Real-Time PCR Reagents	4399461	1802091	Thermo Fisher

4. Sample information

Name of samples	Properties of samples	Number of Sample Delivery Note
CHO-K1/PD1	Cell	BCE20180813YLL01

Procedures and results**1. Prepare samples****Prepare sample lysate**

1. Add 200 µL of Lysis Buffer, then vortex for ~5 seconds to mix.
2. Add:
 - 2 µL of 0.5 M EDTA
 - 18 µL of RNase Cocktail
 Briefly vortex to mix.
3. Incubate at 56°C for 15 minutes.
4. Add 2 µL of Proteinase K, then briefly vortex to mix.
5. Incubate at 56°C for 10 minutes.
6. Incubate at room temperature for 5 minutes.
7. Add 700 µL of Lysis Solution. Vortex for ~5 seconds to mix.

Bind DNA

For each tube of sample lysate:

1. Add 30 µL of Magnetic Particles, then vortex.
2. Add 525 µL of Binding Solution, then invert the tube to mix.
3. Using a vortex adaptor, vortex the tube vertically at medium speed for 5 minutes to capture the nucleic acid.
4. Centrifuge in a microcentrifuge by pressing the short spin button for 15s, then releasing the button. During this time, the microcentrifuge should reach top speed.
5. Place in the Magnetic Stand for 5 minutes.
6. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.

Wash DNA

For each tube of Magnetic Particles pellet (bound DNA):

1. Add 300 µL of Wash Buffer.

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2. Vortex for ~5 seconds.
3. Centrifuge in a microcentrifuge by pressing the short spin button for 15s, then releasing the button. During this time, the microcentrifuge should reach top speed.
4. Place in the Magnetic Stand for 1 minute.
5. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.
6. Repeat steps 1 through 5.
7. Use a P200 pipette to aspirate the residual supernatant from the bottom of the tube, then discard the supernatant.
8. With the lid open, air-dry the Magnetic Particles pellet at room temperature for 5 minutes to remove any remaining ethanol.

Elute DNA

For each sample:

1. Add 100 µL of Elution Buffer.
2. Vortex for ~10 seconds.
3. Incubate at 70°C for 7 minutes. Vortex 2 to 3 times during incubation to ensure complete resuspension of the Magnetic Particles.
4. Centrifuge at top speed for 5 minutes.
5. Place in the Magnetic Stand for 3 minutes.
6. Transfer the eluate to a non-stick 1.5-mL microcentrifuge tube.

2. qPCR**2.1 Prepare qPCR system**

- a. Thaw all kit reagents completely. Life Technologies recommends thawing the positive control at 37 °C for 5 minutes to ensure consistent results.
- b. Vortex, then spin down the reagents.
- c. Prepare the Premix Solution according to the following Table.

Component for premix solution	Volume for one 30-µL reaction (µL)
Power SYBR® Green PCR Master Mix (2X)	15.0
<i>Mycoplasma</i> Real-Time PCR Primer Mix (10X)	3.0
Total premix solution volume	18.0

- d. Mix the Premix Solution by gently pipetting up and down, then cap the tube.
- e. Prepare PCR system

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To prepare...	In each tube or well...
Negative-control reaction	<ul style="list-style-type: none"> Add 18 μL of Premix Solution Add 12 μL of Negative Control (water)
Your unknown sample reaction	<ul style="list-style-type: none"> Add 18 μL of Premix Solution Add 10 μL of unknown sample Add 2 μL of Negative Control (water)
Inhibition-control reaction	<ul style="list-style-type: none"> Add 18 μL of Premix Solution Add 10 μL of unknown sample Add 2 μL of the Discriminatory Positive Control (DPC)
Positive-control reaction	<ul style="list-style-type: none"> Add 18 μL of Premix Solution Add 2 μL of the DPC Add 10 μL of Negative Control (water)

2.2 Set program

Turn on 7500 qPCR instrument, computer and start software, then set new program and select absolute quantification template, followed by setting new test with SYBGR detector and naming as MYCO90, selecting quencher as none, reference dye as ROX and volume as 30 μ L.

2.3 Set cycling parameters

Step	AmpliTa [®] Gold [®] enzyme activation	PCR		Dissociation ^{†‡§}			
	HOLD	Cycle (40 cycles)		Melt			
		Denature	Annea/extend				
Temp	95°C	95°C	60°C	95°C	60°C	95°C	60°C
Time	10 min	15 sec	1 min	15 sec	1 min	15 sec	15 sec

3. Results

Results are listed in Fig.1 and summarized in Tab.1

Fig.1 List of results

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Well	Sample	Target	Task	Quantity	Cr	Tm1	Tm2	Tm3
A1	NTC	Target 1	UNKNOWN		Undetermined	62.3508		
A2	CHO-K1/PD1	Target 1	UNKNOWN		39.148	75.374	62.7177	
A3	CHO-K1/PD1+IC	Target 1	UNKNOWN		25.7441	83.8116	66.9365	
A4	ERC	Target 1	UNKNOWN		30.6525	83.4447	66.0193	
B1	NEG	Target 1	UNKNOWN		Undetermined	62.3508		
B4	POS	Target 1	UNKNOWN		24.9093	84.1785		

Tab.1 Summary of results

Names of samples	Results of mycoplasma tests
NTC	Conformance to Requirements
NEG	
ERC	
POS	
CHO-K1/PD1	No mycoplasma found

Analysis and conclusions

Both of these two controls are of no contamination since CT values of both no template control NC and negative quality control NEG are higher than 36 while Tm values are lower than 75. Results are reliable since CT value of POS is lower than 36 and TM value ≈ 84 .

Note: ΔCT (CT value of sample IC- CT value of PC) lower than 2 means no inhibition factor exists in fq-PCR system. When sample testing result is strong positive (CT value of sample lower than CT value of PC) and TM value higher than 75, inhibition factors will not be considered. Following requirements should be satisfied for sample determination of no mycoplasma contamination: CT value higher than 36 and ΔCT (CT value of sample IC- CT value of PC) lower than 2. When CT value is lower than 36 and TM value higher than 75, samples are indicated as mycoplasma contaminated.

Based on the above analysis, the sample of CHO-K1/PD1 was found no mycoplasma.