Service Report: Order 00001111 Sample

C232. *Mycoplasma Spp.* Decontamination

April 21, 2023

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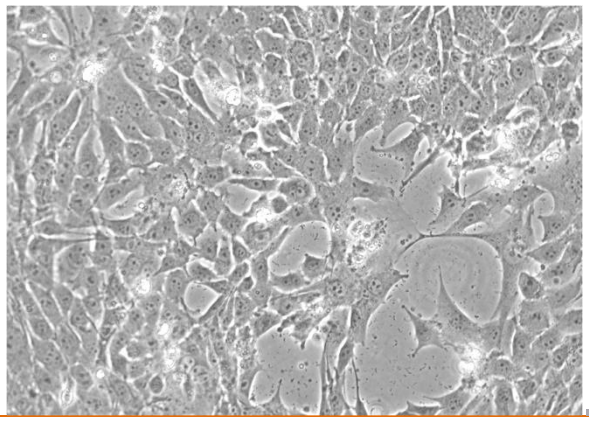
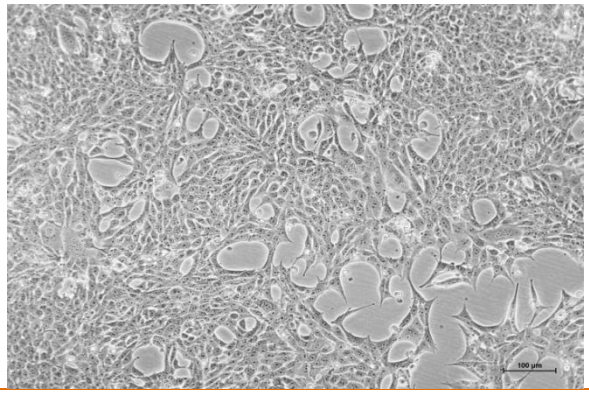
Custom Service Report

Service Summary	
Cat. No.	C232
Service Description	RT-qPCR-based Mycoplasma Detection (**abm** Cat. No. G240): Mycoplasma contamination is indicated by a Ct value <35, with positive control amplification in the 20–23 range.
No. Samples Submitted	1
Deliverable	1 Vial (Cryopreserved): Cell A, Lot#. C26P29ABC

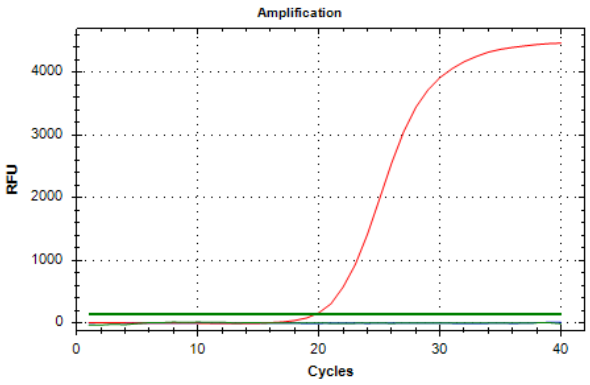
Culture Conditions	
Sample 1	Cell A
Post-freeze Viability	>70%
Cell Count	>1 x 10^6 cells/ml
Growth Properties	Adherent, epithelial
Population Doubling Time (h)	20
Split Ratio	1:2 to 1:4
Propagation Method	For optimal cell culture, we recommend using PriCoat™ T25 Flasks (**abm** Cat. No. G299) or coating your preferred vessels with Applied Cell Extracellular Matrix (**abm** Cat. No. G422). Dulbecco's Modified Eagle Medium (DMEM), High Glucose (**abm** Cat. No. TM500) + 10% FBS + 1% Penicillin/Streptomycin Solution (**abm** Cat. No. G255, 37.0°C, 5% CO_2.

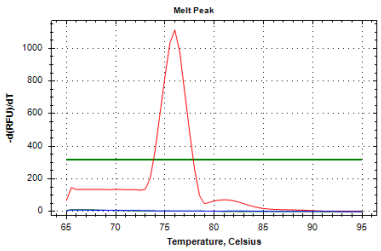
Propagation Details	
Thawing Method	1. Thaw cells quickly in a 37°C water bath while agitating gently (maximum 2 minutes). The vial cap should be kept above the water level to minimize the risk of contamination. 2. Decontaminate the vial by spraying and wiping the exterior of the vial with 70% ethanol. From this point onwards, all operations should be strictly carried out inside a biological safety cabinet using aseptic conditions. 3. Transfer the cell suspension into a 15ml sterile conical tube containing 5ml of pre-warmed, complete growth media. Centrifuge cells at 125xg for 5-7 minutes. 4. Aspirate the supernatant without disturbing the cell pellet. Re-suspend the cell pellet in the recommended pre-warmed, complete growth media and dispense into a T25 culture flask. 5. Incubate the cells at the recommended conditions.
Subculture Method	Volumes given below are for a T75 flask; proportionally increase or decrease the volume as required per culture vessel size. Subculture cells once the culture vessel is 80% confluent. 1. Aspirate the culture media, and add 2-3ml of pre-warmed 0.25% Trypsin-EDTA (**abm** Cat. No. TM050) to the culture vessel. 2. Observe the cells under a microscope to confirm detachment (typically within 2-10 minutes). Cells that are difficult to detach can be put in 37°C, for several minutes to facilitate detachment. 3. Neutralize Trypsin-EDTA by adding an equal volume of the complete growth media into the culture vessel. 4. Transfer the culture suspension into a sterile centrifuge tube, and centrifuge at 125xg for 5 minutes. The actual centrifuge duration and speed may vary depending on the cell type. 5. Aspirate the supernatant, and re-suspend the pellet with pre-warmed fresh complete growth media. Add appropriate aliquots of the cell suspension to new culture vessels, as desired. 6. Incubate the cells at the recommended conditions.
Freezing Medium	CryoGuard™ Freezing Media (**abm** Cat. No. TM078)
Storage Conditions	Vapor phase of liquid nitrogen, or below -130°C.

Cell Morphology and Growth Assessment	
Sample 1: Cell A	
Mycoplasma Contaminated	
Mycoplasma-Free	
Result	Cell A exhibits the correct morphology and is Mycoplasma-Free beyond passage number 8, indicating a successful decontamination service.

Mycoplasma Detection RT-qPCR Results (abm Cat No. G240)		
Sample	**Sample ID**	**Mycoplasma Results**
1	Cell A	-

Legend: + = positive, - = negative.

<table>
<tr><th colspan="4">Mycoplasma Detection RT-qPCR Results (abm Cat No. G240)</th></tr>
<tr><td rowspan="5">RT-qPCR Verification</td><td rowspan="2">Sample</td><td colspan="2">Ct Value</td></tr>
<tr><td>Specification</td><td>Results</td></tr>
<tr><td>NTC</td><td>None Detected</td><td>Not Detected</td></tr>
<tr><td>PTC</td><td>20-23</td><td>19.67</td></tr>
<tr><td>Cell A</td><td>Positive < 35
Negative > 35 or None Detected</td><td>Not Detected</td></tr>
<tr><td colspan="2">Amplification Plot</td><td>Melt Curve</td></tr>
<tr><td colspan="2"> 

Green represents NTC sample
Red represents PTC sample
Blue represents Cell A</td><td> 

Green represents NTC sample
Red represents PTC sample
Blue represents Cell A</td></tr>
<tr><td>Results</td><td colspan="3">Mycoplasma contamination was not detected in Cell A.</td></tr>
</table>

Approval:

Approved by: _____

Approved Personnel ID No.: 0287

Approved Date: April 22, 2023

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Acknowledgement:

*Thank you for choosing **abm** as your service provider. It is our goal to provide you with the best customer experience in the world. Please do not hesitate to contact us should you need further assistance analyzing your data or other services. We are grateful to be a part of your scientific exploration and we look forward to serving you again.*