

# ExCell Bio

## ResiQuant<sup>®</sup> Mycoplasma Detection Kit (Taqman)

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

### User Manual

Catalog Number CRB00-1011S

CRB00-1011

CRB00-1012



## | PRODUCT DESCRIPTION

ResiQuant<sup>®</sup> Mycoplasma Detection Kit (Taqman) is used in combination with the ResiQuant<sup>®</sup> Mycoplasma DNA Isolation Kit to qualitatively detect the presence of mycoplasma contamination in master cell banks, working cell banks, virus seed batches, and cell-derived products.

This kit utilizes fluorescent probe qPCR technology, with primers and probes targeting the highly conserved gene fragments of mycoplasma, covering approximately 200 species. This kit specifically detects mycoplasma and does not cross-react with those closely related bacteria, such as *Lactobacillus*, *Clostridium*, *Streptococcus*, etc., that are required in EP 2.6.7 and common engineered cells like CHO, Vero, HEK293, etc. The performance has been validated according to the requirements for mycoplasma test in EP 2.6.7, JP XVIII and USP <77>. The limit of detection (LOD) reaches 10 CFU/mL NMT, to fulfill the criteria for the substitution of the cell culture method.

This kit incorporates uracil-N-glycosylase (UNG) system, which can effectively digest the possible PCR product contaminants from previous runs reducing the risk of false positives. The dual-channel (FAM+HEX/VIC) detection system enables the kit to have necessary controls integrated into the tests including positive control (PC) and recovery control (RC), therefore the assay sensitivity and sample recovery can be monitored in every test, that is critical for the report by reducing the risk of false negative. The qPCR Mix contains ROX, which plays as a fluorescent reference for optical calibration in the assay, could be helpful especially for the type of instruments like Applied Biosystems<sup>®</sup> Real-Time PCR.

Methodological performance validation is recommended at the beginning assay setup. Limit of detection (LOD), sample matrix interference, and assay specificity as required in EP 2.6.7 are critical for the suitability study. Low-level contamination (e.g. 10 CFU/mL) usually includes a few copies of mycoplasma DNA in the extracts. The statistical distribution of DNA copies in each aliquot is more likely approximate to a binomial distribution. Therefore three-replicate assay manner is strongly suggested in case of releasing test with the confidence level exceeding 95%.

## | PERFORMANCE, APPLICATION AND RESTRICTION

The ResiQuant<sup>®</sup> Mycoplasma Detection Kit (Taqman) is suitable for various types of samples in the

biopharmaceutical process, from intermediates to the final products. In combination with the ResiQuant<sup>®</sup> Mycoplasma DNA Isolation Kit, the kit enables qualitative detection of mycoplasma contamination, with a LOD of 10 CFU/mL.

## | SPECIFICATION, STORAGE AND TRANSPORTATION

### REQUIREMENT

Components	CRB00-1011S (25T)	CRB00-1011 (50T)	CRB00-1012 (100T)
<i>Myc</i> o Positive Control	280 µL	280 µL	280 µL × 2
<i>Myc</i> o Negative Control	280 µL	280 µL	280 µL × 2
<i>Myc</i> o Recovery Control	160 µL	160 µL	160 µL × 2
3× <i>Myc</i> o qPCR Mix	250 µL	500 µL	500 µL × 2

**Storage condition:** -40°C to -18°C.

**Validity:** 12 months under specified storage conditions.

**Transportation:** Dry ice.

**Applicable instrument:** ABI 7500, Bio Rad CFX-96, Agilent Mx3000P, Roche LightCycler 480, etc.

## | EXPERIMENTAL PREPARATION

### Instruments and Reagents Required but Not Included in the Kit

- Real-time qPCR instrument (FAM and HEX/VIC channels must be included. If "reference" option is available, please select "ROX").
- Pipettes and corresponding sterile low retention filter tips.
- Sterile low retention 8-well strips (compatible with the fluorescence quantitative PCR instrument).
- Clean lab coats, disposable gloves, masks, etc.

### Separate Experimental Zones

It is recommended to follow the instruction below to reduce cross-contamination:

- Reagent Preparation Area: Used for preparing 3× *Myc*o qPCR Mix and loading *Myc*o Negative Control.
- Sample Preparation Area: Used for sample nucleic acid extraction, purification, elution, and loading (except *Myc*o Negative Control).

- PCR Amplification Area: For DNA amplification.

Reagent Preparation Area	Sample Preparation Area	PCR Amplification Area
<ul style="list-style-type: none"> <li>❖ 3×<i>Myco</i> qPCR Mix preparation</li> <li>❖ Pipetting of <i>Myco</i> Negative Control to the NTC wells</li> </ul>	<ul style="list-style-type: none"> <li>❖ Extraction and purification of test samples</li> <li>❖ Pipetting of templates to corresponding wells</li> </ul>	<ul style="list-style-type: none"> <li>❖ PCR amplification</li> </ul>

## | EXPERIMENTAL PROCEDURE

Abbr.	Name	Description
PC	Positive Control	Positive control
NTC	No Template Control	Negative control
NCS	Negative Control Sample	Pretreated negative control samples
TS	Test Sample	Pretreated samples to be tested

### DNA Extraction and Purification (Sample Preparation Area)

The ResiQuant® Mycoplasma DNA Isolation Kit is used for sample preparation. For the first-time use, it is strongly recommended to verify the suitability (such as LOD, sample matrix interference, and assay specificity, etc.) during the assay setup.

### PCR Reaction Preparation (Reagent Preparation Area)

- Thaw the 3× *Myco* qPCR Mix at room temperature, vortex, and briefly centrifuge to ensure the reagent collected at the bottom of the tube.
- Determine the number of test:
 
$$\text{Number of Tests} = (1 \text{ NTC} + 1 \text{ PC}) \times 2 + (\text{Number of NCS} + \text{Number of TS}) \times 3$$
- Add 10 µL 3× *Myco* qPCR Mix to each well. (Maintain at 2°C to 8°C prior to reaction initiation).

### Assay Preparation (Sample Preparation Area)

Examples:

Sample Type	3× <i>Myco</i> qPCR Mix	Template
PC	10 µL	20 µL <i>Myco</i> Positive Control
NTC	10 µL	20 µL <i>Myco</i> Negative Control
NCS	10 µL	20 µL Pretreated negative control sample solution
TS	10 µL	20 µL Pretreated test sample solution

Plate Layout Example:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NCS		TS1	TS3	TS5	TS...					PC
B	NTC	NCS		TS1	TS3	TS5	TS...					PC
C		NCS		TS1	TS3	TS5	TS...					
D												
E				TS2	TS4	TS6						
F				TS2	TS4	TS6						
G				TS2	TS4	TS6						
H												

Negative Area
  Sample Area
  Positive Area
  Buffer Area

- Assay preparation is recommended by adding 20 µL template to each corresponding well starting with NTC and sequentially followed by NCS, TS, PC.
- Properly seal the tubes or the 96-well plate with caps or a sealing film, mix and spin briefly before upload the plate to the qPCR machine refer to the plate layout.

### PCR Amplification (PCR Amplification Area)

The following steps are based on the ABI 7500 fluorescence quantitative PCR instrument:

1. Log in to the system, enter the main interface, and click “New Experiment” in the upper-left corner to create a new experiment.
2. Enter the experiment name, select the instrument model as "7500 (96 wells)", experiment type as "Quantitation-Standard Curve", reagents as "qPCR® Reagents", and experiment duration as "Standard".
3. In the "Plate Setup" section, go to "Define Targets and Samples". Set the reporting dye as "FAM", quencher dye as "MGB", add a new reporting dye as "VIC", and quencher dye as "None". Input the sample names.
4. Enter the "Assign Targets and Samples" section and select the target samples and their positions on the 96-well plate.
5. In the lower-left corner, choose "ROX" as the passive reference dye from the drop-down menu.
6. Enter the "Run Method" section and set the reaction volume to 30 µL. Use the following table to set up the reaction program:

**Reaction Program A (for instruments compatible for over 20 steps of amplification program):**

Step		Temperature (°C)	Time (s)	Cycles
1	UNG Incubation	37	300	1
2	Initial Denaturation	95	300	1
3	Denaturation	92	15	2
	Annealing & Extension	65	40	
4	Denaturation	92	15	2
	Annealing & Extension	64	40	
5	Denaturation	92	15	2
	Annealing & Extension	63	40	
6	Denaturation	92	15	2
	Annealing & Extension	62	40	
7	Denaturation	92	15	2
	Annealing & Extension	61	40	
8	Denaturation	92	15	2
	Annealing & Extension	60	40	
9	Denaturation	92	15	2
	Annealing & Extension	59	40	
10	Denaturation	92	15	2
	Annealing & Extension	58	40	
11	Denaturation	92	15	35
	Annealing & Extension (Fluorescence Collection)	57	40	

Mycoplasma Detection Channel: FAM; RC Detection Channel: VIC/HEX

**Reaction Program B (for instruments compatible for less than 20 steps of amplification program):**

Step		Temperature (°C)	Time (s)	Cycles
1	UNG Incubation	37	300	1
2	Initial Denaturation	95	300	1
3	Denaturation	92	15	5
	Annealing & Extension	63	40	
4	Denaturation	92	15	5
	Annealing & Extension	61	40	
5	Denaturation	92	15	6
	Annealing & Extension	59	40	
6	Denaturation	92	15	35
	Annealing & Extension (Fluorescence Collection)	57	40	

Mycoplasma Detection Channel: FAM; RC Detection Channel: VIC/HEX

7. Once all settings are confirmed, click the green button "Start Run" in the upper-right corner to start the assay.
8. When complete the amplification cycles, select "Analysis" on the left side to perform data analysis.
9. In the "Amplification Plot" interface, check the amplification curves to find out the abnormal amplification for further analysis.

### Threshold Setting

Instrument Model	FAM	HEX/VIC
ABI 7500 (with ROX)	0.3 $\Delta$ Rn	0.3 $\Delta$ Rn
Bio-Rad CFX96 (without ROX)	150 RFU	200 RFU

**Note:** For other device models, please verify the threshold line during the assay validation.

### References to The Test Results

Sample	FAM	HEX/VIC	Report
NTC	2 replicates $Ct \geq 30$ or No Ct	$Ct \geq Ct_{PC}+6$ or No Ct	Valid
PC	2 replicates $Ct < 25$ with valid "S" shape amplification	$Ct \leq 24$ with valid "S" shape amplification	Valid
NCS	3 replicates $Ct \geq 30$ or No Ct	$  Ct - Ct_{PC}   \leq 1^*$ is recommended with valid "S" shape amplification	Valid
TS	$Ct < 30$ with valid "S" shape amplification	/	Positive (In case of single positive well, repeat test is recommended.)
	$Ct < 30$ with atypical "S" shape amplification	/	Recommend to repeat the test
	3 replicates $Ct \geq 30$ or No Ct	$  Ct - Ct_{PC}   \leq 1^*$ is recommended with valid "S" shape amplification	Negative
		$Ct > Ct_{PC}+1^*$	Repeat test is recommended to excluding PCR inhibition.

\* Considering cross-laboratory variance, the qualified  $| Ct - Ct_{PC} |$  range can be adjusted based on the validation results with the *Mycoplasma* standard strains.

**Recommendation:** For the validation of the assay sensitivity, prepare 10 CFU/mL *Mycoplasma* spiked sample as positive control sample (PCS), the PCS should be reported as positive with valid "S" shape amplification curve and the  $Ct < 30$  in FAM channel.

**Operation Notes:**

1. Different batches of *Myco* Positive Control and *Myco* Recovery Control shall not be mixed for use.
2. Use disposable gloves, masks, clean lab coats.
3. Use calibrated pipettes.
4. Use sterile low retention filter pipette tips.
5. Use dedicated pipettes, pipette tips and related equipment in different experimental areas.
6. To avoid cross-contamination, be careful when opening and closing the centrifuge tubes.
7. Use dedicated pipettes for NTC, TS and PC to avoid contamination.
8. Load samples sequentially as following, NTC, NCS, TS and PC.
9. Do not put PCR products into reagent preparation area or sample preparation area.
10. Keep PCR reaction tubes sealed after amplification to minimize aerosol contamination.
11. Clean lab benches and instrument surface with 75% alcohol after use.
12. Dispose of used pipette tips by soaking them promptly in 0.1% sodium hypochlorite solution. After the experiment, clean the area and spray 0.1% sodium hypochlorite solution to eliminate aerosol contamination.

**| DISCLAIMER**

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.
2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.