EIV32024E

ExCell Bio

ResiQuant® 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® Mycoplasma Detection Kit (Taqman) is used in combination with the ResiQuant® Mycoplasma DNA Isolation Kit (CAT. CRB00-0031/CRB00-0032) 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 174 species. This kit specifically detects mycoplasma and does not cross-react with those closely related bacteria such as *Lactobacillus*, *Clostridium*, *Streptococcus*, etc which 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® 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.

PERFORMANCE, APPLICATION AND RESTRICTION

The ResiQuant® 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® Mycoplasma DNA Isolation Kit (CAT. CRB00-0031/CRB00-0032), the kit enables qualitative detection of mycoplasma contamination, with a LOD of 10 CFU/mL.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Components	CRB00-1011（50T）	CRB00-1012（100T）	CRB00-1011S（25T）
***Myco* Positive Control**	280 μL	280 μL × 2	280 μL
***Myco* Negative Control**	280 μL	280 μL × 2	280 μL
***Myco* Recovery Control**	150 μL	150 μL × 2	150 μL
3 × *Myco* qPCR Mix	500 μL	500 μL × 2	250 μL

Storage: Store at -40℃ to -18℃.

Shelf Life: 12 months under specified conditions.

Transportation: Dry ice.

Applicable Instruments: ABI 7500, Bio Rad CFX-96, Agilent MX 3000P, Roche et.al.

EXPERIMENTAL PREPARATION

Instruments and Reagents Required but Not Included in the Kit

- Fluorescence quantitative PCR instrument (must have FAM, HEX/VIC channels, and optional ROX channel).
- Pipettes and corresponding sterile low-adsorption filter tips.
- Sterile low-adsorption 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×*Myco* qPCR Mix and loading *Myco* Negative Control;
- Sample Preparation Area: Used for sample nucleic acid extraction, purification, elution, and loading (except NTC);
- PCR Amplification Area: For DNA amplification.

Reagent Preparation Area	Sample Preparation Area	PCR Amplification Area
❖ 3×*Myco* qPCR Mix preparation ❖ Pipetting of *Myco* Negative Control to the NTC wells	❖ Extraction and purification of test samples ❖ Pipetting of templates to corresponding wells	❖ PCR amplification

EXPERIMENTAL PROCEDURE

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

DNA Extraction and Purification (Sample Preparation Area)

The ResiQuant® Mycoplasma DNA Isolation Kit (CAT. CRB00-0031/CRB00-0032) is used for sample preparation. For the first-time use, it is strongly recommended to verify the suitability 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 required based on the validation outcome at the beginning assay setup, for an example as below:

  Number of Tests = (1 NTC+1 PC) × 2 + (Number of NCS + Number of TS) × 3
- Add 10 µL 3× *Myco* qPCR Mix to each well. (Store at 2℃ to 8℃ before reaction).

Assay Preparation (Sample Preparation Area)

Examples:

Sample Type	3× *Myco* qPCR Mix	Template
PC	10 µL	20 µL *Myco* Positive Control
NTC	10 µL	20 µL *Myco* Negative Control
NCS	10 µL	20 µL Negative Control Sample Purified Solution
TS	10 µL	20 µL Test Sample Purified 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 top-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 (℃)	Time (s)	Cycles
1	Pre-digestion	37	300	1
2	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，Fluorescence Collection
	Annealing & Extension	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	Pre-digestion	37	300	1
2	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，Fluorescence Collection
	Annealing & Extension	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 △Rn	0.3 △Rn
Bio-Rad CFX96（without ROX）	150 RFU	200 RFU

References to The Test Results

Sample	FAM	HEX/VIC	Report
NTC	2 replicates Ct ≥ 30 or No Ct	Ct ≥ Ct_{PC}+6 or No Ct	Valid
PC	2 replicates Ct < 25 with valid "S" shape amplification	Ct ≤ 24 with valid "S" shape amplification	Valid
NCS	3 replicates Ct ≥ 30 or No Ct	|Ct-Ct_{PC}|≤ 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 ≥ 30 or No Ct	|Ct-Ct_{PC}|≤ 1* is recommended with valid "S" shape amplification	Negative
		Ct > Ct_{PC}+1*	Repeat test is recommended to excluding PCR inhibition.

* Considering laboratory and sample specificity, the qualified |Ct-Ct_{PC}| range can be adjusted based on the validation results of the Mycoplasma standard strain;

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:

- Use disposable gloves, masks, clean lab coats.
- Use calibrated pipettes.
- Use sterile low adsorption filter pipette tips.
- Use dedicated pipettes, pipette tips and related equipment in different experimental areas.
- To avoid cross-contamination, be careful when opening and closing the centrifuge tubes
- Use dedicated pipettes for NTC, TS and PC to avoid contamination.
- Load samples sequentially as following, NTC, NCS, TS and PC.
- Do not put PCR products into reagent preparation area or sample preparation area.
- Keep PCR reaction tubes sealed after amplification to minimize aerosol contamination;
- Clean lab benches and instrument surface with 75% alcohol after use;
- 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.