Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

XpressMyco qPCR Mycoplasma Detection Kit: Cyclers Programming

LightCycler®2.0

Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature (°C)	95
Incubation Time (min)	2:00
Temperature Transition Rate (°C/s)	20.0
Secondary Target Temperature (°C)	0
Step Size (°C)	0.0
Step Delay (Cycles)	0
Acquisition Mode	None

Program 2: Amplification

Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target Temperature (°C)	95	55	60
Incubation Times (s)	15	30	45
Temperature Transition Rate (°C/s)	20.0	20.0	20.0
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0.0	0.0	0.0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None		
Single	None		

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature (°C)	40
Incubation Time (s)	30
Temperature Transition Rate (°C/s)	20.0
Secondary Target Temperature (°C)	0
Step Size (°C)	0.0
Step Delay (Cycles)	0
Acquisition Mode	None

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Result Reading:

- Select the fluorescence channels 1 and 2
- Click on *Quantification* to generate amplification plots and Ct-values
- The threshold will be generated automatically.
- Samples showing no significant increase in the amplification plot can be considered negative.

ABI Prism® 7500

Detector Settings:

Target Probe:	Reporter – FAM/Quencher – none
Internal Control Probe:	Reporter – HEX/Quencher – none

The "ROX Reference" function needs to be disabled, as no ROX dye is included in the mix. Activate both detectors for each well.

Measure fluorescence during annealing.

Program Step 1: Pre-Incubation

Setting	Hold
Temperature	95°C
Incubation time	3:00 min

Program Step 2: Amplification

Cycles	45
Setting	Cycle
Denaturing	95°C for 30 sec
Annealing	55°C for 30 sec & data reading
Extension	60°C for 45 sec

Result Reading:

- Enter the following basic settings at the right task bar:

Data:	Delta RN vs. Cycle
Detector:	FAM and HEX
Line Color:	Well Color

- Open a new window with the *Graph Settings* by clicking the right mouse button.

  Select the following setting and confirm with ok:

Real Time Settings:	Linear
Y-Axis Post Run Settings:	Linear and Auto Scale
X-Axis Post Run Settings:	Auto Scale
Display Options:	2

- Initiate the calculation of the Ct-values and the graph generation by clicking on Analyze within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no Ct-value can be considered as negative.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Rotor-Gene® 6000 (5-plex)

Program Step 1: Pre-Incubation

Setting	Hold
Hold Temperature	95°C
Hold Time	3 min 0 sec

Please check the correct settings for the filter combination:

Green filter (510): *Mollicutes*

Yellow filter (555): *Internal Control*

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95°C for 5 sec
Annealing	55°C for 30 sec → **acquiring to Cycling A (green and yellow)**
Elongation	60°C for 45 sec
Gain Setting	Automatic (auto gain)
Slope Correct	Activated
Ignore First	Deactivated

Result Reading:

- Open the menu *Analysis*
- Check the required filer set (green and yellow) according to the following table and start data analysis by double-clicking.
- The following windows will appear:

  Quantitation Analysis – Cycling A (green or yellow)

  Quant. Results – Cycling A (green or yellow)

  Standard Curve – Cycling A (green or yellow)
- In the window *Quantitation Analysis*, select first *linear scale* and then *slope correct*

  Threshold setup (not applicable if a standard curve was included in parallel and auto threshold was selected)
  - In window CT Calculation set the threshold value 0-1
  - Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
  - The Ct-values can be taken from the window *Quant. Results.*

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Mx3005P®

- Go to the setup menu, click on “Plate Setup,” check all positions which apply.
- Click on “Collect Fluorescence Data” and check FAM and HEX.
- Corresponding to the basic settings the “Reference Dye” function should be deactivated.
- Specify the type of sample (negative or positive control, sample, standard) at “well type”
- Edit the temperature profile at the “Thermal Profile Design”:

  Segment 1: 95°C for 3 min

  Segment 2:

  Denaturing 95°C for 30 sec

  Annealing 55°C for 30 sec & data collection end

  Extension 60°C for 45 sec

  45 cycles
- At menu “Run Status” select “Run” and start the cycler by pushing “Start”

Data Analysis

- In the window “Analysis” tab on “Analysis Selection/Setup” to analyze the marked positions.
- Ensure that in window “Algorithm enhancement” all options are activated”

  Amplification-based threshold

  Adaptive baseline

  Moving Average
- Click on “Results” and “Amplification Plots: for an automatic threshold
- Read the Ct values at “Text Report”

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

LightCycler®480

Before starting the LC480, make sure that the filter setting is correct:

Instrument I: *Mollicutes* → 533nm, Internal Control → 568 nm

Instrument II: *Mollicutes* → 510nm, Internal Control → 580 nm

Program 1: Pre-Incubation

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature (°C)	95
Incubation time (min)	3:00
Temperature Transition Rate (°C/s)	4.4
Secondary Target Temperature (°C)	0
Step Size (°C)	0.0
Step Delay (Cycles)	0
Acquisition Mode	None

Program 2: Amplification

Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target Temperature (°C)	95	55	60
Incubation time (s)	30	30	45
Temperature Transition Rate (°C/s)	4.4	2.2	4.4
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0.0	0.0	0.0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	Single	None

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature (°C)	40
Incubation time (s)	30
Temperature Transition Rate (°C/s)	2.2
Secondary Target Temperature (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Biorad CFX 96™

Run Setup Protocol Tab

Click **Create New** to open the *Protocol Editor* to create a new protocol.

Select any step in either the graphical or text display – the step becomes highlighted in blue.

Click the temperature or well time to directly edit the value.

1	95.0°C for 3:00
2	95.0 °C for 0:30
3	55.0 °C for 0:30
	+ Plate Read
4	60.0 °C for 0:45
5	GOTO 2 .44 more times
	END

Run Setup Plate Tab

Click **Create New** to open the Plate Editor to create a new plate.

Use the **Scan Mode** dropdown menu in the Plate Editor toolbar to designate the data acquisition mode to be used during the run.

Important!!! Select the *All Channels* mode.

Click **Select Fluorophores** to indicate the fluorophores that will be used in the run. Choose FAM for the detection of mycoplasma amplification and HEX for monitoring the amplification of the internal control. Within the plate diagram, select the wells to load.

Quantification Tab

The amplification chart displays traces of the relative fluorescence collected from each well at every cycle of the run. Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under the amplification chart. Select FAM to display data of mycoplasma detection and select HEX to display internal control amplification data.

Data Analysis

The software uses two modes for quantification cycle determination. Select **Settings** from the menu bar and select **Baseline Subtracted Curve Fit** as baseline setting and **Single Threshold** mode as Cq Determination Mode. In the Single Threshold Mode, click and drag the threshold line to manually position the line. Adapt the threshold line to the initial linear section of the positive control reaction.

Samples showing no Ct-value can be considered negative.

Trademarks