applied biosystems

ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit

Catalog Number 4444415

Publication Number 4445235

Revision E

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision history: Pub. No. 4445235 (English)

Revision	Date	Description
E	26 June 2018	Updated template and legal information. Reorganized content. Added screen shots of example results using AccuSEQ™ software.
D	March 2011	Baseline for this revision history.

Contents

Product information

Product description

The ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit is a TaqMan®-based Real-Time PCR kit for highly sensitive detection of MMV in cell culture research samples. The kit contains TaqMan® probe and primer mix, TaqMan® Environmental Master Mix 2.0, negative control, and a dual-purpose positive control. Assays for MMV, Vesivirus, and Mycoplasma can be set up and run in the same plate.

Sample preparation

We recommend using the PrepSEQ™ sample preparation procedure in the *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799), or the *PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus User Guide* (Pub. No. 4465957). These procedures allow you to detect approximately 0.1–0.2 TCID[50] per mL of sample. (TCID[50] is the median tissue culture infective dose, that is, the amount of a pathogenic agent that produces pathological change in 50% of cell cultures inoculated.)

Note: TCID[50] can vary depending on accuracy of cell culture assay.

Kit specificity

The ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit can detect all known MMV strains:

- MMVi
- MMVp
- MMVm
- MMVc

The kit does not detect other genera or cell-line DNA. The following table shows species that the kit is not designed to detect.

Organism	Strain/source
Human	Promega™
Mouse	Novagen™
Rat	Novagen™
Chinese Hamster Ovary (CHO)-K1	Lofstrand
Streptococcus pneumoniae	ATCC
Saccharomyces cerevisiae	ATCC
Staphylococcus enterica	ATCC

Organism	Strain/source
Staphylococcus aureus	ATCC
Escherichia coli	ATCC

Contents and storage

Table 1 ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit (Cat. No. 4444415)

Contents	Cap color	Amount	Storage
Box 1, SEQ Real-Time PCR Core Kit			
2× Environmental Master Mix 2.0		2 × 0.825 mL	Protect from light. –25°C to –15°C until first use. After first use, store at 2–8°C.
Negative Control (water)		1.0 mL	
Box 2, MMV Real-Time PCR Assay Mix			
10× MMV Assay Mix		0.300 mL	Protect from light. –25°C to –15°C
Box 3, MMV Discriminatory Positive/Extraction Control			
MMV Positive Control, 1,000 copies/µL		0.700 mL	–25°C to –15°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instruments; choose one:	
7500 Fast Real-Time PCR System with AccuSEQ™ software	Contact your local sales representative
(Optional) 7500 Real-Time PCR System	
Consumables	
Disposable gloves	MLS
Aerosol-resistant pipette tips	MLS

Item	Source
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.2-mL well	4306737[1]
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, 20 plates; for use with 7500 Fast Real-Time PCR System	4346906
MicroAmp™ Optical Adhesive Film Kit, 20 covers, 1 applicator, 1 optical cover compression pad	4313663
MicroAmp™ Optical Adhesive Film, 25 or 100 covers	4360954, 25 covers 4311971, 100 covers
Microfuge tubes, 1.5-mL, RNase-free, nonstick	AM12450

[1] Not recommended for use with the 7500 Fast system. For 7500 Fast system reactions, use Cat. No. 4346906.

Workflow

IMPORTANT! For information on how to avoid PCR contamination, see Appendix C, "Good laboratory practices".

Prepare the sample (page 8)

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Prepare the kit reagents and premix solution (page 8)

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Methods

Prepare the sample

See the *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799) or the *PrepSEQ™ Sample Preparation Kits Protocol* for details on sample preparation.

Prepare the kit reagents and premix solution

1. Thaw all kit reagents completely.
2. Vortex briefly, then spin down the reagents.
3. Label a microcentrifuge tube for the premix solution.
4. Prepare the Premix Solution according to the following table.

   IMPORTANT! Use a separate pipette tip for each component.

Component for Premix Solution	Volume for one 30-µL reaction	Volume for four 30-µL reactions[1]
2× Environmental Master Mix 2.0	15.0 µL	66.0 µL
10× MMV Assay Mix	3.0 µL	13.2 µL
Negative Control (water)	2.0 µL	8.8 µL
Total Premix Solution Volume	**20 µL**	**88 µL**

[1] Includes 10% excess to compensate for pipetting errors.

5. Mix the Premix Solution by gently pipetting up and down, then cap the tube.

Prepare the PCR reactions

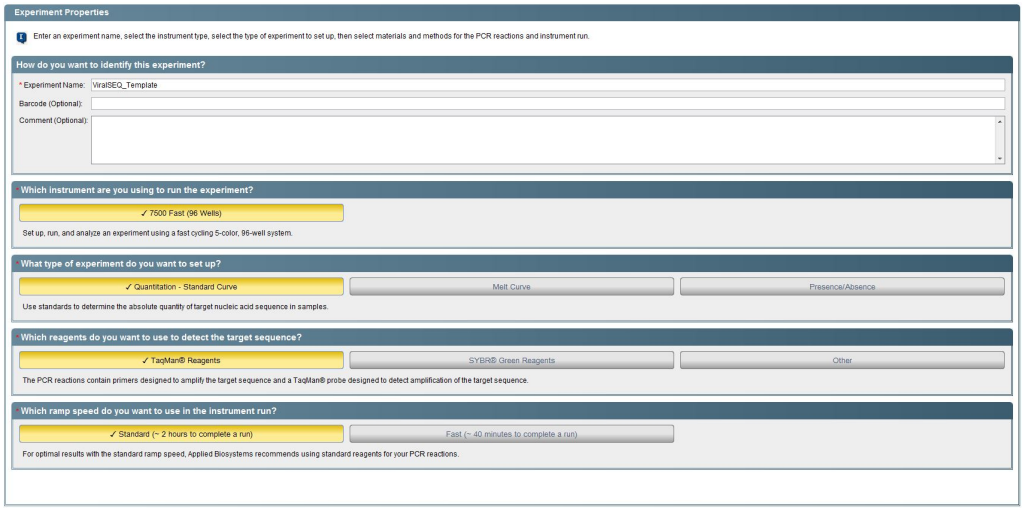
1. Dispense the following into each well to be used, gently pipetting at the bottom of the well.

To prepare...	Combine in each tube or well...
Negative-control reaction	• 20 µL of Premix Solution • 10 µL of Negative Control (water)
Unknown sample reaction	• 20 µL of Premix Solution • 10 µL of unknown sample
Positive-control reaction	• 20 µL of Premix Solution • 2 µL of MMV Positive Control • 8 µL of Negative Control (water)

2. Seal the plates. For details, see "Seal the plates" on page 24.

Create a plate document

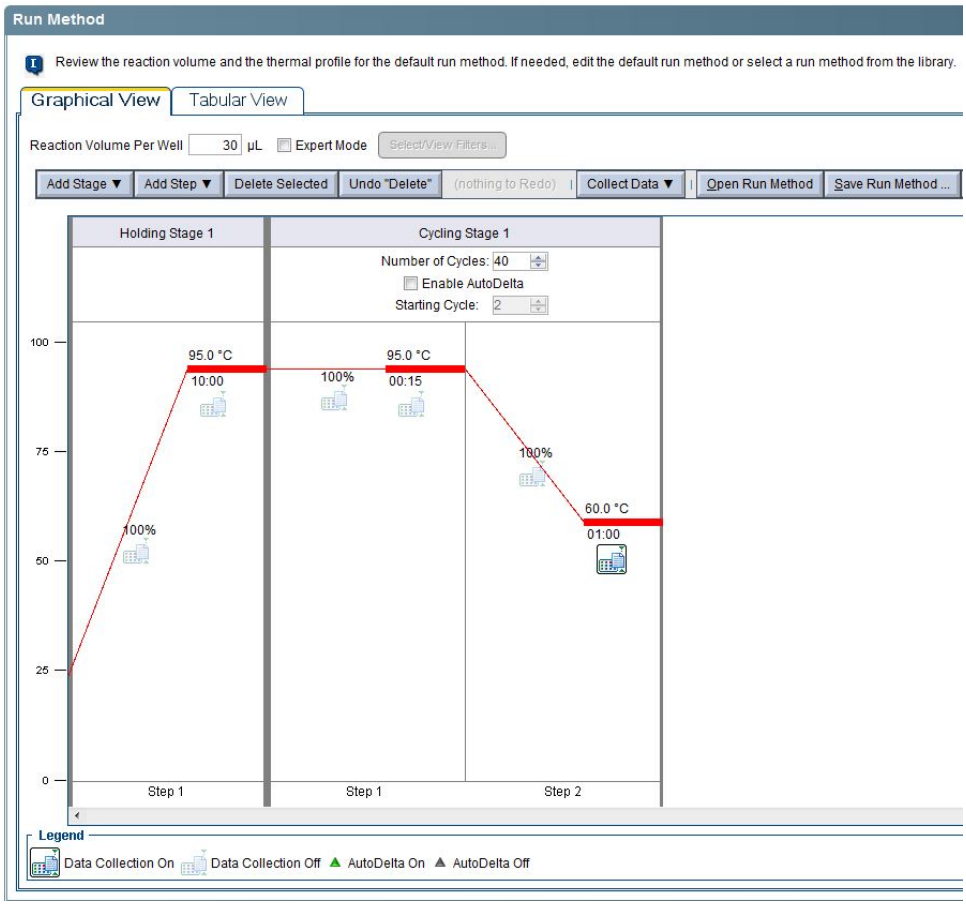
Create a plate document In the AccuSEQ™ software



1. In the home screen, select **Create Custom Experiment**.
2. In the **Experiment** name field, enter a unique name for the experiment.
3. Specify experiment information:
   a. Select experiment type **Quantitation – Standard Curve**.
   b. Select reagents **TaqMan® Reagents**.
   c. Select ramp speed **Standard**.
4. In the Plate Setup screen, select the **Define Targets and Samples** tab.

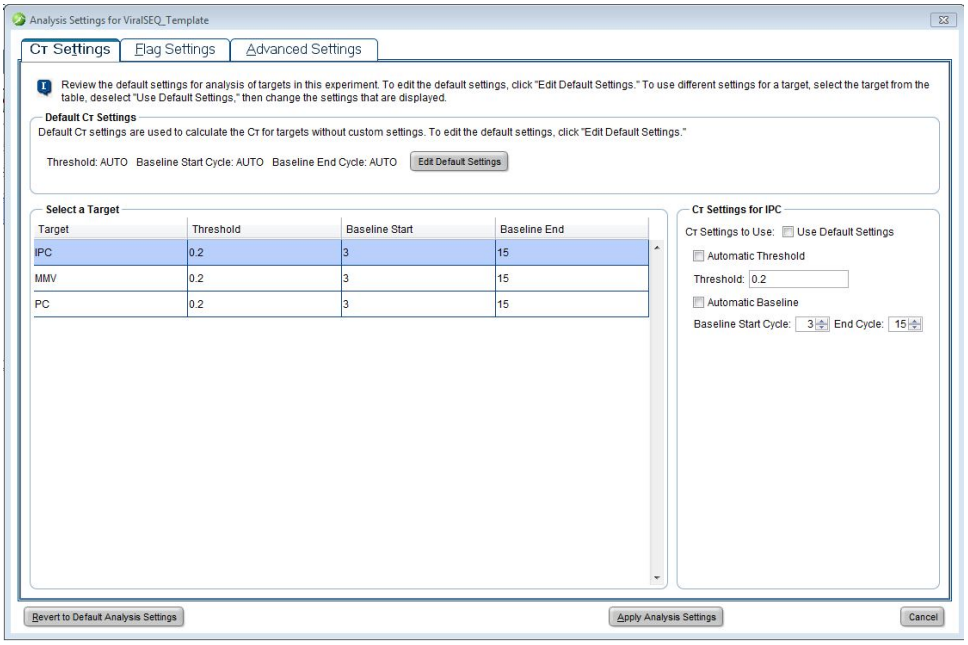
5. Specify Target Information:
   a. Click **Add New Target**.
   b. Enter ***MMV*** in the target name field.
   c. Select reporter **FAM™** and quencher **NFQ_MGB**.
   d. Select a color for this target.
6. Specify PC Target Information:
   a. Click **Add New Target**.
   b. Enter **PC** in the target name field.
   c. Select reporter **VIC™** and quencher **NFQ_MGB**.
   d. Select a color for this target.
7. Specify IPC Target Information:
   a. Click **Add New Target**.
   b. Enter **IPC** in the target name field.
   c. Select reporter **NED™** and quencher **NFQ_MGB**.
   d. Select a color for this target.
8. Verify that **ROX™** is selected as the Passive Reference.
9. Click **Add New Sample**.
10. In the **Assign Targets and Samples** tab, for each sample, click to highlight the appropriate well, then click to assign the sample and target to the well.

11. In the **Run Method** screen, set the **Reaction Volume Per Well** to **30 µL**.



12. In the Analysis Settings window, enter the following settings, then click **OK**:
    a. Deselect **Use Default Settings**.
    b. Deselect **Automatic Threshold**.
    c. In the Threshold field, enter **0.2**.

d. Select **Manual Baseline** at **Baseline Start Cycle 3** to **End Cycle 15**.



13. To generate an EDT template file, select **File ▸ Save as**, confirm that the file is named "ViralSEQ_Template", then select **Save as a template file** in the drop-down list and close the template plate document.

    Note: You can reuse the plate template document whenever you run the assay.

Run the plate

1. In the toolbar, select **File ▸ Open**, navigate to the **ViralSEQ_Template** file (created in "Create a plate document" on page 9), then click **Open**.
2. In the Plate Name field, enter **ViralSEQ_** ***date of Assay***, then click **Finish**.
3. Make any necessary changes to the test sample labels.
4. Select **Save As** to save the new experiment as an EDS experiment file.
5. Load the plate into the instrument.
6. Click **Start Run**.
7. Select a run screen (**Amplification** plot, **Temperature** plot, or **Run method**) to monitor the progress of the run.

Analyze the results

After the qPCR run is finished, use the following general procedure to analyze the results:

1. In the toolbar, select **Analysis** ▸ **Analysis Settings**.
2. Click Analyze (**Analyze**).
3. Select **Analysis** ▸ **QC Summary** in the left panel of the screen. Review the flag summary.
4. Select **File** ▸ **Export**. In the Export Data menu, select file type ***.xls**. Click **Start Export**.
5. Select **File** ▸ **Print Report** to generate a hard copy of the experiment, or click **Print Preview** to view and save the report as a *.pdf or *.html file.

View and verify the results

View the amplification plot

1. In the **Results** tab, select the **Amplification Plot** tab.
2. In the drop-down list in the Detector field at the top-right corner of the plot screen, select the appropriate detectors.
3. Evaluate the results for wells according to Table 2.

   Note: The recommended acceptance criteria is subject to your own validation.

Table 2 Recommended acceptance criteria

Sample Type	FAM™ C_t value[1]	VIC™ C_t value[2]	NED™ signal	Call
PCR Negative Control (NTC)	Undetermined	Undetermined	Present	Pass, no control or target DNA detected (Example: "Negative controls" on page 30)
PCR Positive Control (PTC) (PTC, 2,000 copies/reaction)	$C_t \leq 32$	$C_t \leq 32$	Present	Pass, positive control DNA detected (Example: "Positive controls" on page 29)
Extraction Negative Control (ENC) (Sample DNA extraction)	Undetermined	Undetermined	Present	Pass, no spiked control or target DNA detected (Example:"Negative controls" on page 30)
Extraction Positive Control (EPC) (Sample DNA extraction)	$C_t \leq 39.99$	$C_t \leq 39.99$	Present	Pass, spiked positive control DNA detected (Example: "Positive controls" on page 29)

Sample Type	FAM™ C_t value[1]	VIC™ C_t value[2]	NED™ signal	Call
Test sample	Undetermined	Undetermined	Present	Negative for MMV (Example: "Test samples: Negative result" on page 33)
Test sample	$C_t \leq 39.99$	Undetermined	Present	Positive for MMV (Example:"Test samples: Positive result" on page 32)

[1] The average FAM™ C_t for 20 copies of MMV genomic DNA is 36.7 ± 0.7

[2] The average VIC™ C_t for 10 copies of MMV Positive Control is 37.1 ± 0.3.

Interpret results

Examine the wells for these characteristics:

- **IPC signal (NED™ dye)** should be present in all wells. For most instruments, the IPC in all wells should have a C_t between 28 and 36 at the manual C_t threshold of 0.2.

  If there is a large amount of viral DNA due to competition, the IPC signal can be inhibited or absent in test samples . In a test sample, samples with viral DNA competition show:

  – Low C_t for the target-specific signal (FAM™ dye)
  – $C_t > 36$ or Undetermined for the IPC signal (NED™ dye)
  – No positive control signal (VIC™ dye)

  If there is viral inhibition and the target-specific signal (FAM™ dye) is present, the IPC signal (NED™ dye) can be ignored and the sample considered positive for the presence of MMV.
- **Negative controls:** PCR negative control (NTC) and extraction negative control (ENC)

  Only IPC signal (NED™ dye), should be present in the negative controls. If either VIC™ dye or FAM™ dye is present, the control shows contamination and it is necessary to repeat the experiment with freshly prepared samples and reagents.
- **Positive controls:** PCR positive control (PTC) and extraction positive control (EPC)

  Target-specific signal (FAM™ dye), positive control signal (VIC™ dye), and IPC signal (NED™ dye) should be present in the positive controls. If they are not, repeat the experiment with freshly prepared samples and reagents.
- **Test samples (Unknowns):** If the only signal detected is IPC (NED™ dye), the test sample is negative for the presence of MMV. If both IPC (NED™ dye) and target-specific (FAM™ dye) signals are detected, the test sample is positive for the presence of MMV. If positive control signal (VIC™ dye) is present, the test sample shows contamination with the positive control and it is necessary to repeat the experiment with freshly prepared samples and reagents.

The information in the following examples applies to results generated on any Applied Biosystems™ Real-Time PCR System.

Examples of results

The figures in this section show examples of results that meet or do not meet criteria for interpretation that are set in our laboratories. Users should interpret results according to their laboratory protocols.

See Appendix B, “Troubleshooting“ if signals do not meet the appropriate criteria.

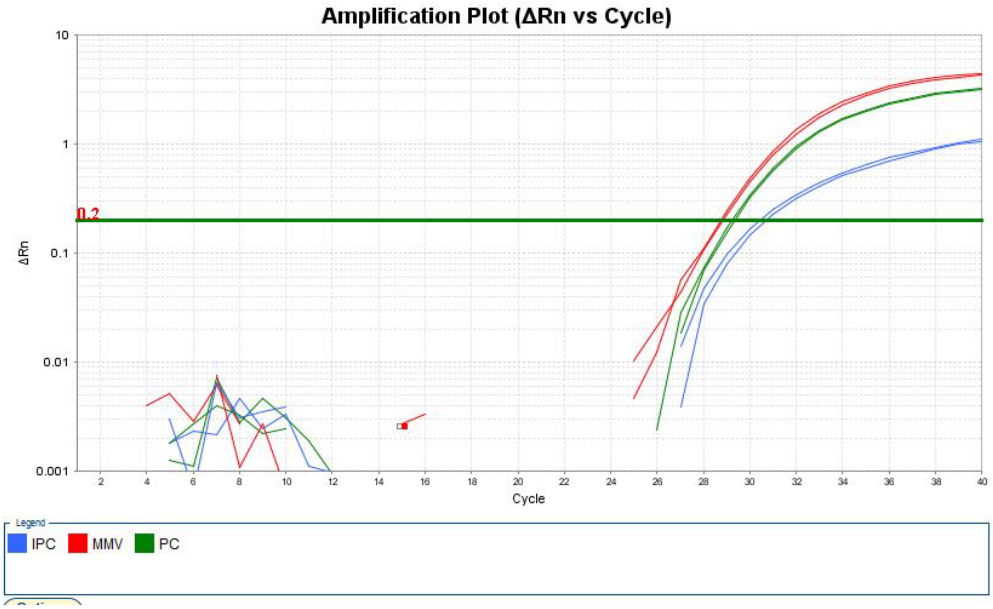
Amplification plots were generated on the 7500 Fast Real-Time PCR System with AccuSEQ™ Software v2.1.1.

Positive controls

The criteria for positive controls are:

- FAM™ dye (red, MMV)–$C_t \leq 39.99$
- VIC™ dye (green, PC)–$C_t \leq 39.99$
- NED™ dye (blue, IPC)–$C_t \leq 39.99$

The amplitude curves and reports below meet these criteria.



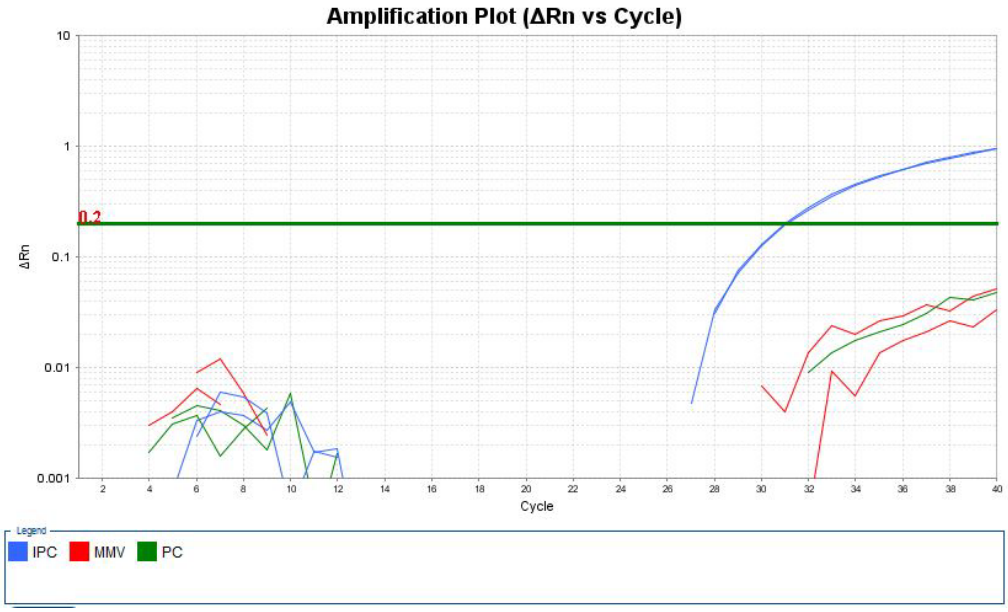
Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Negative controls

The criteria for negative controls are:

- FAM™ dye (red, MMV)–C_t undetermined
- VIC™ dye (green, PC)–C_t undetermined
- NED™ dye (blue, IPC)–$C_t \leq 39.99$

The amplitude curves and reports below meet these criteria.



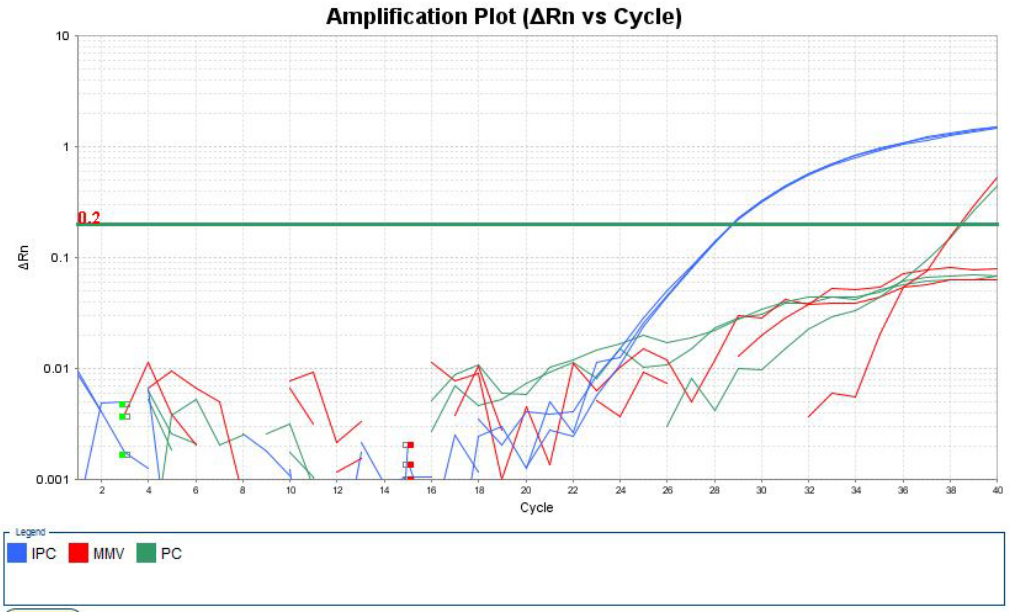
Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Cross contamination with the positive control

FAM™ and VIC™ dye signal in the unknown sample or negative control reaction indicates contamination by the Positive Control. If FAM™ and VIC™ dye signal appears in these wells, the reactions should be repeated.

- Positive control in negative control wells:
  - FAM™ dye (red, MMV)–Present
  - VIC™ dye (green, PC)–Present
- Positive control in test sample:
  - FAM™ dye (red, MMV)–Present
  - VIC™ dye (green, PC)–Present

In these cases, we recommend repeating the experiment with freshly prepared sample and reagents.



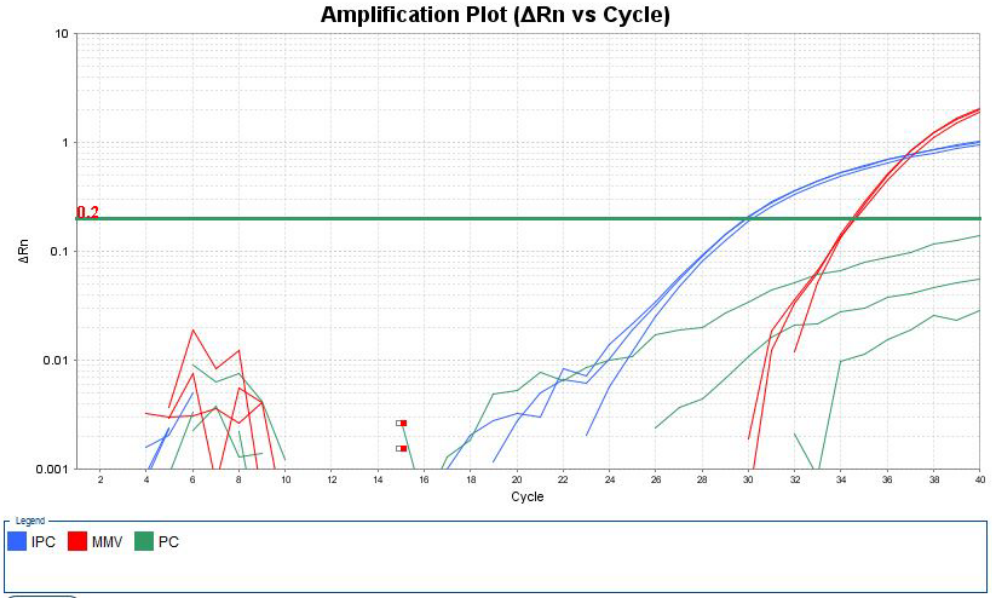
Test samples: Positive result

The criteria for positive results are:

- FAM™ dye (red, MMV)–$C_t \leq 39.99$
- VIC™ dye (green, PC)–C_t none, with no amplification
- NED™ dye (blue, IPC)–C_t Present

The IPC signal can be inhibited or absent if there is a large amount of viral DNA due to competition.

The amplitude curve and report below meet our criteria for positive results.



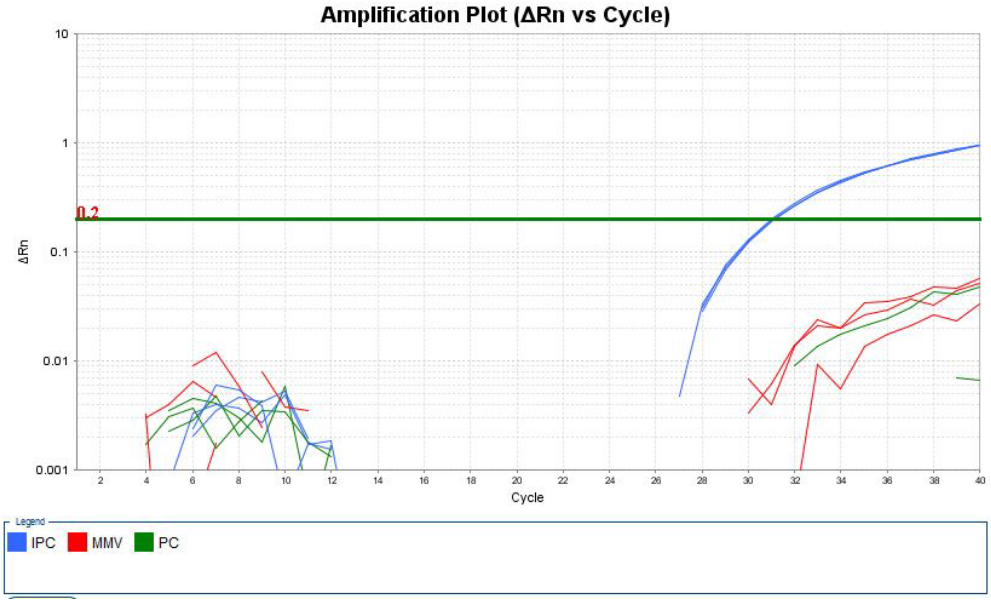
Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Test samples: Negative result

The criteria determined in our laboratories for negative results are:

- FAM™ dye (red, MMV)–C_t undetermined
- VIC™ dye (green, PC)–C_t undetermined
- NED™ dye (blue, IPC)–Present

The amplitude curve and report below meet our criteria for negative results.



Note: Users should determine criteria for their laboratory according to their laboratory protocols.

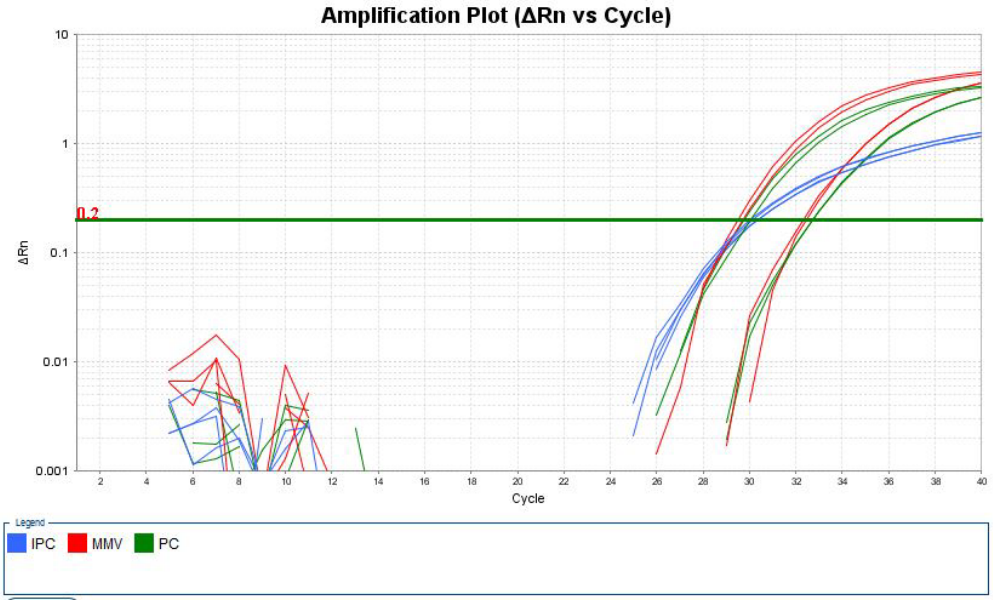
Test samples: Positive extraction control

The criteria for extraction spiked with positive control DNA are:

- FAM™ dye (red, MMV)–C_t Present
- VIC™ dye (green, PC)–$C_t \leq 39.99$
- NED™ dye (blue, IPC)–C_t Present

The IPC signal can be inhibited or absent if there is a large amount of viral DNA due to competition.

The amplitude curve and report below meet our criteria for positive results.



Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Test samples: Inconclusive result

If the amplitude curves for all of the dyes show an undetermined signal, then the result is inconclusive. We recommend repeating the experiment with freshly prepared reagents and freshly prepared re-purified sample.

A Use the kit with 7500 System SDS Software v1.4 or later

Prepare the sample

See the *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799) or the *PrepSEQ™ Sample Preparation Kits Protocol* for details on sample preparation.

Prepare the kit reagents and premix solution

1. Thaw all kit reagents completely.
2. Vortex briefly, then spin down the reagents.
3. Label a microcentrifuge tube for the premix solution.
4. Prepare the Premix Solution according to the following table.

IMPORTANT! Use a separate pipette tip for each component.

Component for Premix Solution	Volume for one 30-µL reaction	Volume for four 30-µL reactions[1]
2× Environmental Master Mix 2.0	15.0 µL	66.0 µL
10× MMV Assay Mix	3.0 µL	13.2 µL
Negative Control (water)	2.0 µL	8.8 µL
Total Premix Solution Volume	**20 µL**	**88 µL**

[1] Includes 10% excess to compensate for pipetting errors.

5. Mix the Premix Solution by gently pipetting up and down, then cap the tube.

Prepare the PCR reactions

1. Dispense the following into each well to be used, gently pipetting at the bottom of the well.

To prepare...	Combine in each tube or well...
Negative-control reaction	• 20 µL of Premix Solution • 10 µL of Negative Control (water)
Unknown sample reaction	• 20 µL of Premix Solution • 10 µL of unknown sample
Positive-control reaction	• 20 µL of Premix Solution • 2 µL of MMV Positive Control • 8 µL of Negative Control (water)

2. Seal the plates. For details, see "Seal the plates" on page 24.

Prepare the plate document

1. During setup of the plate document, in the **Assay** drop-down list, select **Absolute Quantification**.

2. Select **FAM™**, **VIC™**, and **NED™** detectors with:
   - Quencher Dye set to **none** or **Non Fluorescent**
   - Passive Reference set to **ROX™**

3. Set thermal-cycling conditions as shown in the following table and figure.

Step	**AmpliTaq Gold™ enzyme activation**	**PCR**	
	HOLD	Cycle (40 cycles)	
		Denature	Anneal/extend
Temp	95°C	95°C	60°C
Time	10 min	15 sec	1 min

   - Set **Sample Volume** to **30 µL**
   - For the 7500 Fast system, set **Run Mode** to **Standard 7500**
   - Set Data Collection to **Stage 2, Step 2 (60.0°C 1:00)**

- For details, see the:
  - *Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve* (Pub No. 4347825)

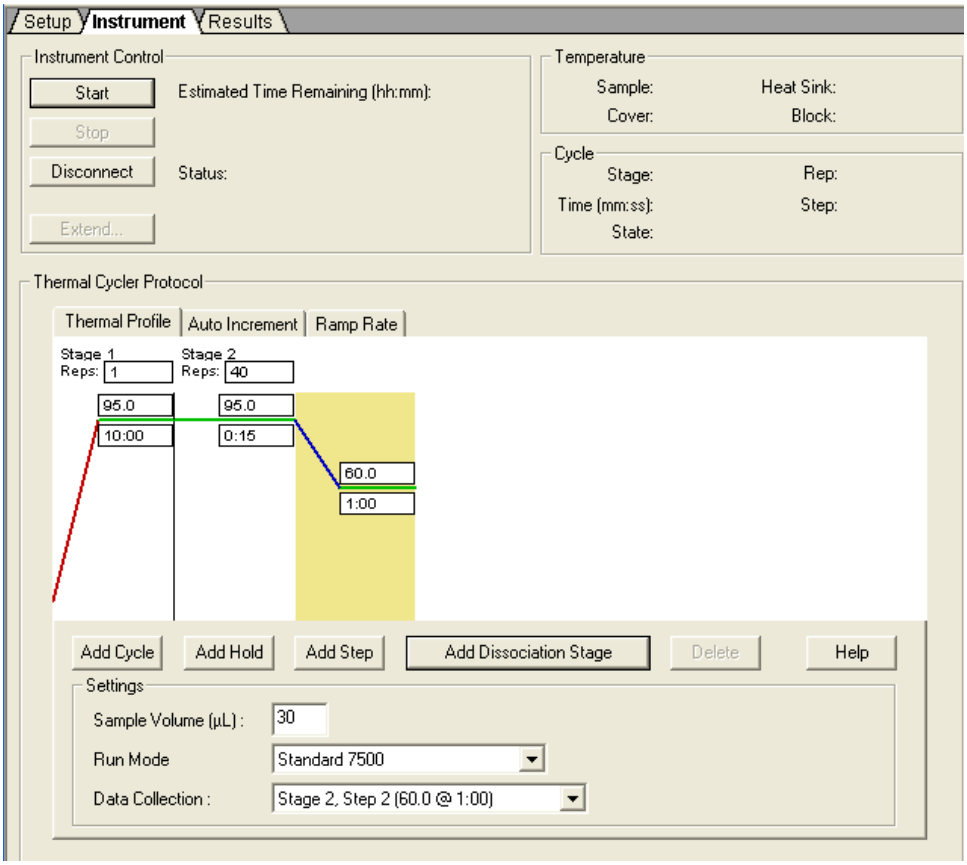
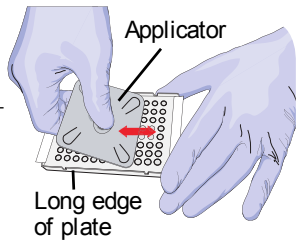


Figure 1 Plate document settings

Seal the plates

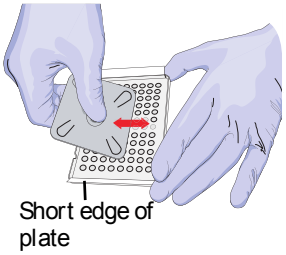
1. Place an optical adhesive cover on the plate, then rub the flat edge of the applicator back and forth along the **long** edge of the plate.



IMPORTANT! Apply significant downward pressure on the applicator to completely seal the wells. Pressure is required to activate the adhesive on the optical cover.

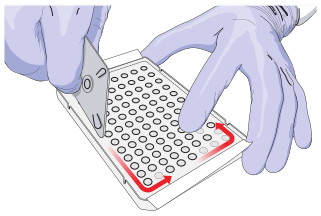
2. Rub the flat edge of the applicator back and forth along the **short** edge (width) of the plate.

3. Rub the edge of the applicator horizontally and vertically between all wells.



4. Rub the edge of the applicator around all outside edges of the plate using small back and forth motions to completely seal around the outside wells.

5. Briefly spin down the plate using a centrifuge with a plate adapter.



IMPORTANT! Make sure that the reagents (and no bubbles) are in the bottom of the wells.

Perform PCR

On an Applied Biosystems™ Real-Time PCR System:

1. Open the plate document that corresponds to the reaction plate you created on "Prepare the plate document" on page 23.
2. Load the reaction plate into the real-time PCR system.
3. Start the run.

Analyze the plate document

For specific instructions on analyzing your results, refer to the user guide for your real-time PCR instrument.

1. Open the plate document.
2. Select the wells to analyze.
3. In the **Results** tab, click **Analysis**. Select **Analyze** in the drop-down list.
4. In the **Analysis Settings** dialog box, select **Manual Baseline**.
   - C_t: **Manual**
   - Start (cycle): **3**
   - End (cycle): **15**
   - Threshold: **0.2**

View and verify the results

View the amplification plot

1. In the **Results** tab, select the **Amplification Plot** tab.
2. In the drop-down list in the Detector field at the top-right corner of the plot screen, select the appropriate detectors.
3. Evaluate the results for wells according to Table 3.

   Note: The recommended acceptance criteria is subject to your own validation.

Table 3 Recommended acceptance criteria

Sample Type	FAM™ C_t value[1]	VIC™ C_t value[2]	NED™ signal	Call
PCR Negative Control (NTC)	Undetermined	Undetermined	Present	Pass, no control or target DNA detected (Example: "Negative controls" on page 30)
PCR Positive Control (PTC) (PTC, 2,000 copies/reaction)	$C_t \leq 32$	$C_t \leq 32$	Present	Pass, positive control DNA detected (Example: "Positive controls" on page 29)
Extraction Negative Control (ENC) (Sample DNA extraction)	Undetermined	Undetermined	Present	Pass, no spiked control or target DNA detected (Example:"Negative controls" on page 30)
Extraction Positive Control (EPC) (Sample DNA extraction)	$C_t \leq 39.99$	$C_t \leq 39.99$	Present	Pass, spiked positive control DNA detected (Example: "Positive controls" on page 29)
Test sample	Undetermined	Undetermined	Present	Negative for MMV (Example: "Test samples: Negative result" on page 33)
Test sample	$C_t \leq 39.99$	Undetermined	Present	Positive for MMV (Example:"Test samples: Positive result" on page 32)

[1] The average FAM™ C_t for 20 copies of MMV genomic DNA is 36.7 ± 0.7

[2] The average VIC™ C_t for 10 copies of MMV Positive Control is 37.1 ± 0.3.

Interpret results

Examine the wells for these characteristics:

- **IPC signal (NED™ dye)** should be present in all wells. For most instruments, the IPC in all wells should have a C_t between 28 and 36 at the manual C_t threshold of 0.2.

  If there is a large amount of viral DNA due to competition, the IPC signal can be inhibited or absent in test samples . In a test sample, samples with viral DNA competition show:

  – Low C_t for the target-specific signal (FAM™ dye)

  – $C_t > 36$ or Undetermined for the IPC signal (NED™ dye)

  – No positive control signal (VIC™ dye)

  If there is viral inhibition and the target-specific signal (FAM™ dye) is present, the IPC signal (NED™ dye) can be ignored and the sample considered positive for the presence of MMV.
- **Negative controls:** PCR negative control (NTC) and extraction negative control (ENC)

  Only IPC signal (NED™ dye), should be present in the negative controls. If either VIC™ dye or FAM™ dye is present, the control shows contamination and it is necessary to repeat the experiment with freshly prepared samples and reagents.
- **Positive controls:** PCR positive control (PTC) and extraction positive control (EPC)

  Target-specific signal (FAM™ dye), positive control signal (VIC™ dye), and IPC signal (NED™ dye) should be present in the positive controls. If they are not, repeat the experiment with freshly prepared samples and reagents.
- **Test samples (Unknowns):** If the only signal detected is IPC (NED™ dye), the test sample is negative for the presence of MMV. If both IPC (NED™ dye) and target-specific (FAM™ dye) signals are detected, the test sample is positive for the presence of MMV. If positive control signal (VIC™ dye) is present, the test sample shows contamination with the positive control and it is necessary to repeat the experiment with freshly prepared samples and reagents.

The information in the following examples applies to results generated on any Applied Biosystems™ Real-Time PCR System.

Examples of results

The figures in this section show examples of results that meet or do not meet criteria for interpretation that are set in our laboratories. Users should interpret results according to their laboratory protocols.

SeeAppendix B, “Troubleshooting“ if signals do not meet the appropriate criteria.

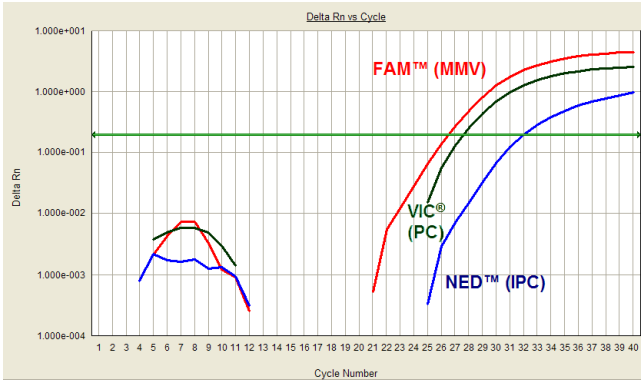
Amplification plots were generated on the 7500 Fast Real-Time PCR System with SDS Software Software v1.4.

Positive controls

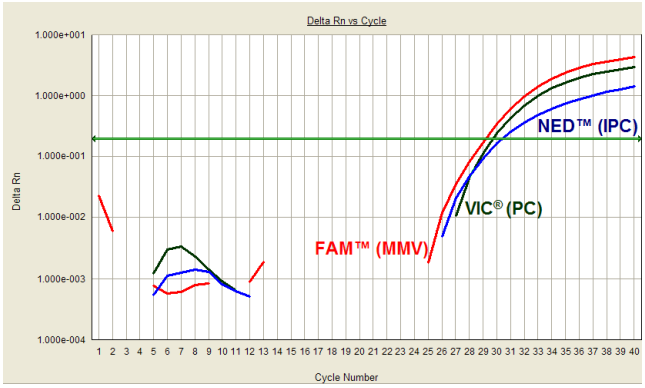
The criteria for positive controls are:

- FAM™ dye (red, MMV)–$C_t \leq 39.99$
- VIC™ dye (green, PC)–$C_t \leq 39.99$
- NED™ dye (blue, IPC)–$C_t \leq 39.99$

The amplitude curves and reports below meet these criteria.



PCR Positive Control (2,000 copies of positive control)



Extraction Positive Control (EPC)

Well	Sample Name	Detector	Task	Ct
H7	Positive Control	MMV	Unknown	26.4739
H7	Positive Control	PC	Unknown	27.5483
H7	Positive Control	IPC MMV	Unknown	32.0536

Well	Sample Name	Detector	Task	Ct
G3	EPC	FAM-MMV	Unknown	29.1461
G3	EPC	VIC-MMV PC	Unknown	29.6802
G3	EPC	NED-IPC	Unknown	30.4158

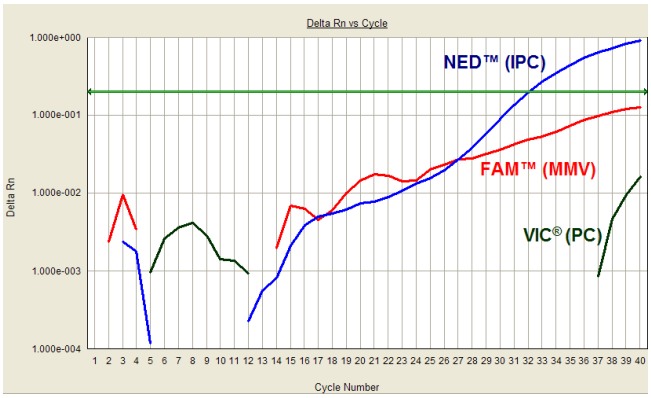
Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Negative controls

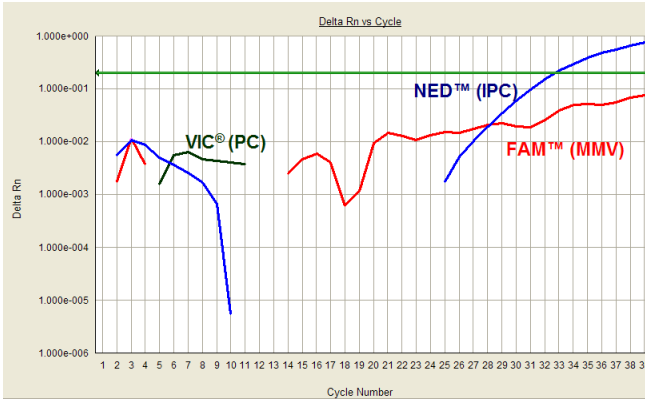
The criteria for negative controls are:

- FAM™ dye (red, MMV)–C_t undetermined
- VIC™ dye (green, PC)–C_t undetermined
- NED™ dye (blue, IPC)–$C_t \leq 39.99$

The amplitude curves and reports below meet these criteria.



Non-template Control (NTC)



Extraction Negative Control (ENC)

Well	Sample Name	Detector	Task	Ct
A1	NTC	MMV	NTC	Undet.
A1	NTC	PC	NTC	Undet.
A1	NTC	IPC MMV	Unknown	32.0636

Well	Sample Name	Detector	Task	Ct
A3	BEC	FAM	Unknown	Undet.
A3	BEC	VIC	Unknown	Undet.
A3	BEC	NED	Unknown	32.7444

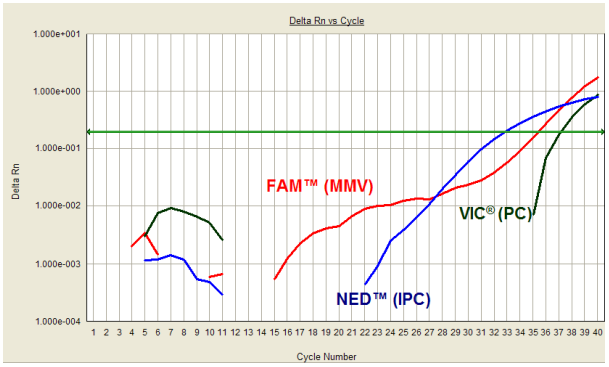
Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Controls indicating contamination

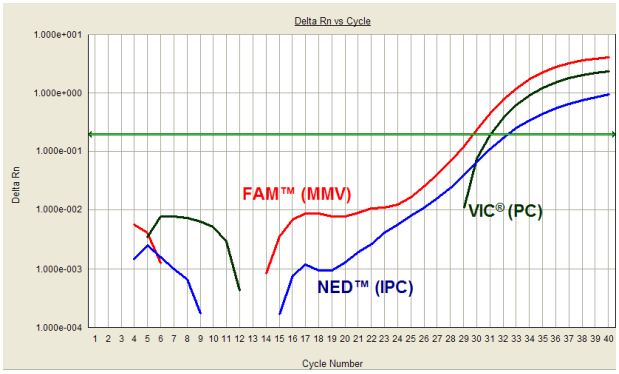
FAM™ and VIC™ dye signal in the unknown sample or negative control reaction indicates contamination by the Positive Control. If FAM™ and VIC™ dye signal appears in these wells, the reactions should be repeated. The amplitude curves and reports below show results that do not meet our criteria.

- Positive control in negative control wells:
  - FAM™ dye (red, MMV)–Present
  - VIC™ dye (green, PC)–Present
- Positive control in test sample:
  - FAM™ dye (red, MMV)–Present
  - VIC™ dye (green, PC)–Present

In these cases, we recommend repeating the experiment with freshly prepared sample and reagents.



Positive control in ENC



Positive control in test sample

Well	Sample Name	Detector	Task	Ct
E8	Negative Control	MMV	Unknown	35.388
E8	Negative Control	PC	Unknown	37.1439
E8	Negative Control	IPC MMV	Unknown	32.8761

Well	Sample Name	Detector	Task	Ct
G9	Sample B	MMV	Unknown	29.6897
G9	Sample B	PC	Unknown	31.0512
G9	Sample B	IPC MMV	Unknown	32.3624

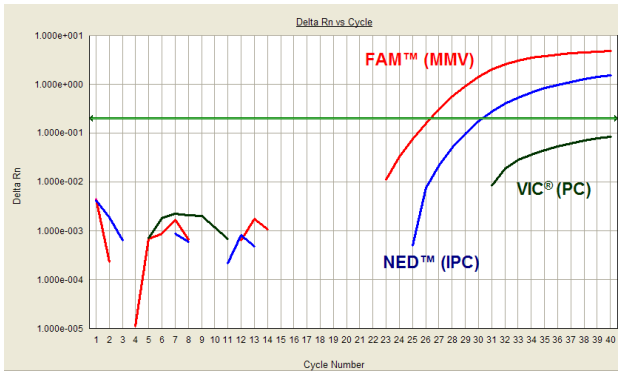
Test samples: Positive result

The criteria for positive results are:

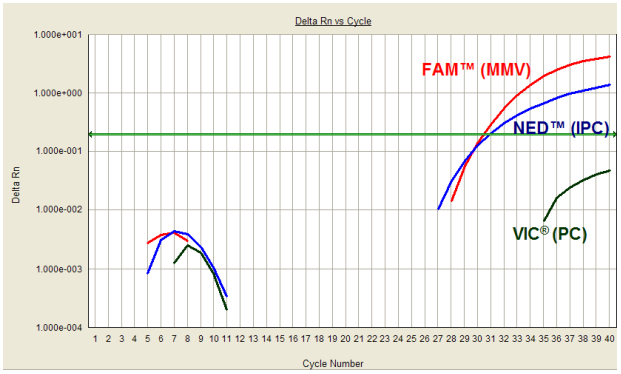
- FAM™ dye (red, MMV)–$C_t \leq 39.99$
- VIC™ dye (green, PC)–C_t undetermined
- NED™ dye (blue, IPC)–C_t Present

The IPC signal can be inhibited or absent if there is a large amount of viral DNA due to competition.

The amplitude curve and report below meet our criteria for positive results.



MMV-positive result



MMV-positive result

Well	Sample Name	Detector	Task	Ct
C1	Sample 1	FAM-MMV	Unknown	26.2838
C1	Sample 1	VIC-MMV PC	Unknown	Undet.
C1	Sample 1	NED-IPC	Unknown	30.2462

Well	Sample Name	Detector	Task	Ct
H1	Sample 2	FAM-MMV	Unknown	30.4179
H1	Sample 2	VIC-MMV PC	Unknown	Undet.
H1	Sample 2	NED-IPC	Unknown	30.9542

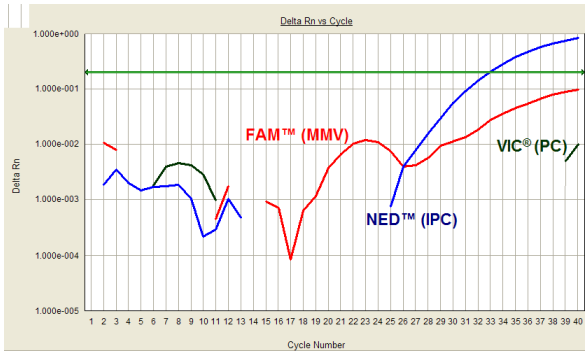
Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Test samples: Negative result

The criteria determined in Applied Biosystems™ laboratories for negative results are:

- FAM™ dye (red, MMV)–C_t undetermined
- VIC™ dye (green, PC)–C_t undetermined
- NED™ dye (blue, IPC)–Present

The amplitude curve and report below meet our criteria for negative results.



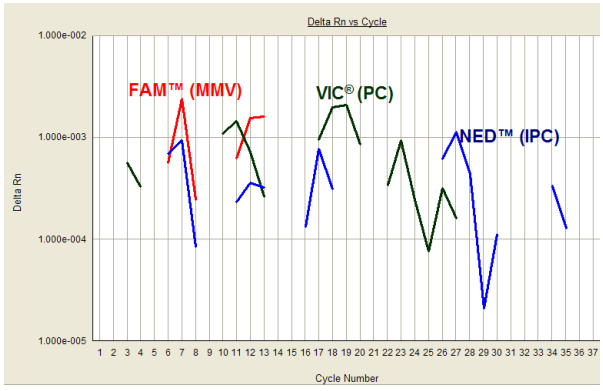
MMV-negative result

Well	Sample Name	Detector	Task	Ct
A1	Sample A	FAM	Unknown	Undet.
A1	Sample A	VIC	Unknown	Undet.
A1	Sample A	NED	Unknown	32.8782

Note: Users should determine criteria for their laboratory according to their laboratory protocols.

Test samples: Inconclusive result

The amplitude curve and report below show undetermined signal for all dyes. This result is inconclusive. We recommend repeating the experiment with freshly prepared reagents and freshly prepared re-purified sample.



Inconclusive result

Well	Sample Name	Detector	Task	Ct
E5	Sample 5	FAM-MMV	Unknown	Undet.
E5	Sample 5	VIC-MMV PC	Unknown	Undet.
E5	Sample 5	NED-IPC	Unknown	Undet.

B

Troubleshooting

Observation	Possible cause	Recommended action
No MMV target-specific signal (FAM™ dye) is detected in PCR positive control and/or extraction positive control wells.	FAM™ detector not selected.	Make sure FAM™ detector is selected in the plate document (see "Prepare the plate document" on page 23). Repeat the analysis with the FAM™ detector selected.
No positive control signal (VIC™ dye) is detected in PCR positive control and extraction positive control wells.	VIC™ detector not selected.	Make sure VIC™ detector is selected in the plate document (see "Prepare the plate document" on page 23). Repeat the analysis with the VIC™ detector selected.
	Pipetting error (no positive control added).	Repeat the assay. Make sure to add positive control into all positive-control wells.
IPC signal C_t > 36 or undetermined	Inhibition of PCR.	Repeat the sample preparation, then repeat the assay. If PCR inhibition continues, contact your FAS, or dilute the sample (for example, 1:5 or 1:10) to dilute inhibitors.
No IPC signal is detected, but target-specific signal is detected	High copy number of target DNA resulting in preferential amplification of the target-specific DNA.	Report sample as positive.
No IPC signal is detected	NED™ dye detector not selected.	Make sure NED™ detector is selected in the plate document (see "Prepare the plate document" on page 23). Repeat the analysis with the NED™ detector selected.
Target-specific signal or positive control signal is detected in negative control wells.	Carryover contamination.	1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative control still continues to show contamination, contact Technical Support.

Observation	Possible cause	Recommended action
Amplification plot crosses the threshold but no target-specific signal is amplified.	Probe hydrolysis or non-optimal baseline setting.	View amplification plots for the affected wells using Rn vs. C_t to confirm no up-tick in amplification. Change baseline settings. Check manual 3–15 or autobaseline. Contact Technical Support.
No dye signal in test sample well	Error in preparation.	Repeat the sample preparation and perform a new PCR reaction, using freshly prepared sample and reagents.

C Good laboratory practices

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Avoiding false positives due to cross-contamination

To avoid false positives due to cross-contamination:

- Prepare and close all negative control and unknown sample tubes before pipetting the positive control.
- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative control, unknown, and positive control samples.

Plate layout suggestions

- For each plate row, dispense in sequence from left to right the: negative controls, unknown samples, and positive controls (at the end of the row or column).
- Place positive controls in one of the outer columns.
- If possible, separate all samples from each other by at least one well; if space is limiting, place at least one well between unknown samples and controls.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit Quick Reference	4445236
PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide	MAN0016799
PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection Quick Reference	MAN0017291
PrepSEQ™ Sample Preparation Kits User Guide	4401253
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve	4347825

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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