BCR-ABL1<sup>IS</sup> MR3 Assay<sup>TM</sup>

For Quantitative RT-PCR Analysis of BCR-ABL1

**Product Description and User Manual**

Catalog Number 901-0032

Store at -30°C to -15°C

For Research Use Only. Not for use in Diagnostic Procedures.
NOTICE TO PURCHASER: LIMITED LICENSE

This product is for research use only and is licensed to the user under MolecularMD intellectual property only for the purchaser’s internal research. The purchase of this product includes a license to use this product solely for the purchaser’s own internal research and may not be used for any other commercial use, including without limitation repackaging or resale in any form. This product is sold under a license from the ELITech Group. The purchase of this product does not convey to the purchaser any license to perform the Polymerase Chain Reaction “PCR” process under any third party rights.
MolecularMD® BCR-ABL1^IS MR3 Assay™

Catalog # 901-0032

Product Description and User Manual

Product Overview
A. Introduction ........................................................................................................... 4
B. Test Description .................................................................................................... 5
C. Intended Use ......................................................................................................... 5
D. Handling and Storage .......................................................................................... 5
E. Assay Design ......................................................................................................... 6
F. Reagents Required and Not Provided ..................................................................... 6
G. Instrument Recommended ..................................................................................... 6
H. Components .......................................................................................................... 6

Protocol Overview

Important Notes for Optimal Results ................................................................. 7
A. Reagent Preparation: Reconstitution Procedure .................................................. 7
B. Sample RNA Preparation .................................................................................... 8
C. Experimental Design ........................................................................................... 8
D. Standard Assay Preparation and Run ................................................................. 9

Results and Analysis .............................................................................................12

Run Evaluation Criteria .......................................................................................13

Troubleshooting Guide .........................................................................................14

References ............................................................................................................14
Product Overview

A. Introduction

The MolecularMD BCR-ABL1<sup>IS</sup> MR3 Assay™ provides accurate and sensitive quantitation of BCR-ABL and ABL levels. In the test, BCR-ABL1 levels are measured and normalized using the ABL gene product as an endogenous reference. The test is performed using a one-step RT-qPCR protocol wherein the reverse transcription and quantitative PCR reactions are performed in the same well. Quantitation is achieved using DNA calibration standards and linear regression analysis. BCR-ABL transcript levels are measured and reported in relation to the ABL transcript as an endogenous reference, resulting in the BCR-ABL/ABL ratio percent.

In November 2009, the Expert Committee on Biological Standardization of the World Health Organization (WHO) endorsed the first International Genetic Reference Panel for quantitation of BCR-ABL translocation by RT-qPCR. The MolecularMD BCR-ABL1<sup>IS</sup> MR3 Assay™ provides a conversion factor (CF) traceable to the WHO reference panel for reporting the BCR-ABL/ABL ratio percentage on the International Scale (WHO IS).<sup>1,2</sup>

The MolecularMD BCR-ABL1<sup>IS</sup> MR3 Assay™ provides:

International Scale Results

The conversion factor assigned to the assay allows for converting test sample BCR-ABL/ABL ratios to the International Scale (IS).

Control RNAs

Low, High, and Negative Control RNAs are assayed in parallel with test samples to ensure test accuracy. The Low Control RNA is targeted to MR3 (MMR) or 0.10% IS BCR-ABL/ABL. The High Control RNA is targeted to MR1 or 10% IS BCR-ABL/ABL. The Negative Control RNA is negative for BCR-ABL and positive for ABL providing a test for false positive amplification of BCR-ABL. (See Table 8)

One-Step Reverse Transcription-qPCR protocol

This protocol combines the reverse transcription and qPCR reactions both streamlining the protocol and increasing assay sensitivity.

Calibrators

A calibrator series for both BCR-ABL and ABL targets is provided in a dilution series. This calibrator series is used for establishing standard curves based on the known concentration of the calibrators, permitting precise quantitation of the BCR-ABL and ABL levels for each test sample.

TaqMan® Probe Technology

TaqMan® probe technology provides superior test accuracy and sensitivity compared to traditional probe designs.
B. Test Description

This test is used for determining BCR-ABL levels in a test sample comprised of total RNA extracted from the test specimen. BCR-ABL levels are determined by one-step RT-qPCR using the SuperScript®III Platinum® One-Step Quantitative RT-PCR System (Life Technologies). A forward PCR primer in BCR exon 13 and a reverse primer in ABL exon 2 allows for the amplification of both e13a2 and e14a2 p210 BCR-ABL transcripts (table below). ABL serves as the endogenous control gene. Both TaqMan® probes are labeled with fluorescein (FAM). Standard curves of known amounts of BCR-ABL and ABL allow calculation of the ratio of BCR-ABL to ABL in the test sample. The test result is expressed as a fraction of BCR-ABL to that of the control gene ABL (% BCR-ABL/ABL).

To obtain a value on the IS, the BCR-ABL/ABL ratio determined is multiplied by a conversion factor (CF). The CF for the MolecularMD BCR-ABL1 IS MR3 Assay™ is 0.98. If desired, the CF may also be determined for each laboratory by following the instructions for the NIBSC WHO International Standard 1st WHO International Genetic Reference Panel.

Figure 1: Schematic of the primer and probe locations of the assay. The arrows represent the primers and the small squares represent the BCR-ABL probe (blue) and ABL probe (red).

TABLE 1 - Primer/Probe Sets

<table>
<thead>
<tr>
<th>Target</th>
<th>Forward Primer</th>
<th>TaqMan Probe</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL</td>
<td>BCR exon 13</td>
<td>ABL exon 2</td>
<td>ABL exon 2</td>
</tr>
<tr>
<td>ABL</td>
<td>ABL exon 3</td>
<td>ABL exons 3-4</td>
<td>ABL exon 4</td>
</tr>
</tbody>
</table>

C. Intended Use

The MolecularMD BCR-ABL1 IS MR3 Assay™ is intended for use in research studies for accurate quantification of BCR-ABL and ABL levels in biological samples. This product is not intended for diagnostic use. The assay is intended for use by personnel trained in and familiar with quantitative RT-PCR technology.

D. Handling and Storage

The MolecularMD BCR-ABL1 IS MR3 Assay™ will remain stable under correct storage conditions until the expiration date printed on the component label. Store the product, before and after reconstitution, at -30°C to -15°C in a constant-temperature freezer.

PRECAUTION: The Calibrators should be stored and used in an area separate from the test sample and assay preparation area in order to prevent cross contamination.
E. Assay Design
The MolecularMD BCR-ABL1\textsuperscript{IS} MR3 Assay™ is designed for testing 24 duplicate test samples in concert with Calibrators, No Template Controls (NTCs), and the set of three Control RNAs. Sufficient Calibrators and Control RNAs are provided to run each set in three separate 96-well plates.

F. Reagents Required and Not Provided
The MolecularMD BCR-ABL1\textsuperscript{IS} MR3 Assay™ is optimized and validated for use with the Invitrogen SuperScript\textsuperscript{®}III Platinum\textsuperscript{®} One-Step Quantitative RT-PCR System (Life Technologies catalog #11732-020) and RNaseOUT\textsuperscript{®} (Life Technologies catalog #10777-019). Nuclease-free water for making Master Mixes and NTCs is not provided.

G. Instrument Recommended
Applied Biosystems\textsuperscript{®} 7500 Fast or Applied Biosystems\textsuperscript{®} 7500 Fast Dx (see Table 6; pg. 10)

H. Components

- Control RNAs (Refer to Table 8; pg. 13):
  - Control RNA - HIGH, 0.1 µg/µL (140 µL reconstituted)
  - Control RNA - LOW (MMR), 0.1 µg/µL (140 µL reconstituted)
  - Control RNA - NEG, 0.1 µg/µL (140 µL reconstituted)

- BCR-ABL + ABL Calibrators, 6 vials:
  - $3\times 10^5$: 300,000 copies/10 µL, 100 µL total volume provided
  - $3\times 10^4$: 30,000 copies/10 µL, 100 µL total volume provided
  - $3\times 10^3$: 3,000 copies/10 µL, 100 µL total volume provided
  - $3\times 10^2$: 300 copies/10 µL, 100 µL total volume provided
  - $3\times 10^1$: 30 copies/10 µL, 100 µL total volume provided (BCR-ABL wells only)
  - $3\times 10^0$: 3 copies/10 µL, 135 µL total volume provided (BCR-ABL wells only)

- Primer/Probe Set Mixes, 2 vials (Pre-mixed PCR primers and probes):
  - BCR-ABL P/P Set: 25X concentration (120 µL reconstituted)
  - ABL P/P Set: 25X concentration (120 µL reconstituted)

- Additional:
  - Nuclease-Free Water: For Components; (1000 µL total volume provided); not for Master Mixes or NTCs
Protocol Overview

The Control RNAs and Primer/Probe Set Mixes are provided as freeze dried materials that must be reconstituted prior to running the assay. To perform the assay, separate Master Mixes containing all of the reaction components are prepared for BCR-ABL and ABL reactions. The master mix is dispensed into reaction wells, the RNA or water (NTCs) is added, and the inoculated wells sealed. After the plate is moved to a separate area, the Calibrators are added to the plate and the wells sealed. The plate is placed into the instrument.

Important Notes for Optimal Results

✓ The Calibrators should be stored and used in an area separate from the test sample and assay preparation areas in order to prevent cross contamination.
✓ The Control RNAs and Primer/Probe Set Mixes must be reconstituted following the instructions below prior to running the assay. This reconstitution step is critical for optimal results.
✓ Keep test samples and Control RNAs on ice when in use.
✓ Accurately determine the total RNA concentration of the test sample before starting the test procedure.
✓ Vortex the Control RNAs and Calibrators according to reconstitution procedure below prior to use.
✓ Always pipette the sample into the master mix by pipetting up-and-down thoroughly.
✓ Duplicate testing of Control RNAs and test samples is strongly recommended.

A. Reagent Preparation: Reconstitution Procedure

❖ Control RNAs
  1. Centrifuge the vials for 30 seconds in a standard microcentrifuge at maximum speed.
  2. Add 140 µL of Nuclease-Free Water (provided) to each vial, centrifuge briefly and then incubate on ice for 60 minutes. Note: this step is important to sufficiently rehydrate and equilibrate the target RNA.
  3. Vortex for 30 seconds then centrifuge the vials as before.
  4. Use immediately or store at -30°C to -15°C in a constant temperature freezer.

❖ Primer/Probe Set Mixes
  1. Centrifuge the vials for 30 seconds in a standard microcentrifuge at maximum speed.
  2. BCR-ABL P/P Set vial (red cap): Add 120 µL of the Nuclease-Free Water provided to the BCR-ABL P/P Set vial, centrifuge briefly and then incubate on ice for 30 minutes.
  3. ABL P/P Set vial (blue cap): Add 120 µL of the Nuclease-Free Water provided to the ABL P/P Set vial, centrifuge briefly and then incubate on ice for 30 minutes.
4. Vortex for 30 seconds then centrifuge the vials as in Step 1.
5. Use immediately or store at -30°C to -15°C.

B. Sample RNA Preparation
Accurately determine the total RNA concentration of the test sample before starting the test procedure. Preparation of the RNA test sample should be performed using a validated extraction procedure. Assay performance is optimal using 1 µg of total RNA at a concentration of 0.1 µg/µL. The Control RNAs are at 0.1 µg/µL concentration.

C. Experimental Design
Each test sample and Control RNA should be assayed in duplicate in separate BCR-ABL and ABL reactions. An example plate layout for testing eight (8) samples is provided below. Note that Calibrator range for ABL quantification is 300,000 to 300 copies.

Example Plate:
Example assay layout for eight (8) test samples in duplicate using a 96-well plate
Wells containing BCR-ABL Primer/Probe reactions are colored **RED**
Wells containing ABL Primer/Probe reactions are colored **BLUE**

<table>
<thead>
<tr>
<th>TABLE 2 - Example Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CALIBRATORS</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>H</td>
</tr>
</tbody>
</table>

**NTC = No Template Control**
D. Standard Assay Preparation and Run

Procedure

1. Thaw the Calibrators to room temperature (15°C to 25°C). In the meantime, prepare the Master Mixes.

2. Thaw the test sample RNA, Control RNAs, Primer/Probe Sets, and reaction components on ice. The reaction components include RNaseOUT® and the components of the SuperScript®III Platinum® One-Step Quantitative RT-PCR System.

3. Determine the number of reactions needed. The Master Mix contains all of the reaction components except the RNA or DNA template. Determine the number of reactions needed for each Master Mix using the tables below; “n” is the number of test samples. Duplicate testing of Control RNAs and test samples is strongly recommended.

<table>
<thead>
<tr>
<th>Samples &amp; Controls</th>
<th>Number of Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test RNA Samples</td>
<td>n samples in duplicate: 2n reactions total</td>
</tr>
<tr>
<td>Calibrators</td>
<td>Single: 3e5, 3e4, 3e3, 3e2 Duplicate: 3e1 Quadruplicate: 3e0 10 reactions total</td>
</tr>
<tr>
<td>Control RNAs</td>
<td>Each Control in duplicate: 6 reactions total</td>
</tr>
<tr>
<td>NTC (water)</td>
<td>NTC in duplicate: 2 reactions total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples &amp; Controls</th>
<th>Number of Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test RNA Samples</td>
<td>n samples in duplicate: 2n reactions total</td>
</tr>
<tr>
<td>Calibrators</td>
<td>Single 3e5, 3e4, 3e3, 3e2: 4 reactions total</td>
</tr>
<tr>
<td>Control RNAs</td>
<td>Each Control in duplicate: 6 reactions total</td>
</tr>
<tr>
<td>NTC (water)</td>
<td>NTC in duplicate: 2 reactions total</td>
</tr>
</tbody>
</table>

4. Prepare separate BCR-ABL and ABL Master Mixes for the number of reactions needed using Table 5. It is important to mix the solutions well before use. A single reaction volume is 25 µL (15 µL Master Mix + 10 µL of each test sample RNA, RNA Control, Water or Calibrator).
TABLE 5 - Master Mixes

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme Mix</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>2X Reaction Mix</td>
<td>12.5 µL</td>
</tr>
<tr>
<td>Primer/Probe Set</td>
<td>1 µL</td>
</tr>
<tr>
<td>ROX Reference Dye (1:10 dilution)*</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>RNaseOUT®</td>
<td>0.5 µL</td>
</tr>
</tbody>
</table>

*Prior to using 0.5 µL ROX Reference Dye in the Master Mixes, follow instructions in the package insert for the Invitrogen SuperScript® III Platinum® One-Step Quantitative RT-PCR System (Life Technologies catalog #11732-020) for preparation of a 1:10 dilution of the ROX Reference Dye. A sufficient volume of 1:10 ROX dilution must be prepared to allow addition of 0.5 µL ROX Reference Dye per reaction into the Master Mixes.

TABLE 6 - ROX dye

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Amount of ROX per 25 µL Reaction</th>
<th>Final ROX Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems® 7500 Fast; Applied Biosystems® 7500 Fast Dx</td>
<td>0.5 µL</td>
<td>50 nM</td>
</tr>
</tbody>
</table>

5. Mix the Master Mix gently by pipetting the solution up and down three (3) times.
6. Add 15 µL of the Master Mix to each well according to the plate map.
7. Add 10 µL of the same Nuclease-Free water used in the Master Mix into the NTC wells.
8. Vortex the thawed Control RNAs for 30 seconds and briefly centrifuge. It is important to mix the Control RNAs completely before use.
9. Add 10 µL of each Control RNA (provided at [0.1µg/µL] in the kit) into the appropriate wells. It is important to carefully dispense the RNA directly into the Master Mix and mix by pipetting the solution up and down three (3) times.
10. If test sample RNA is frozen, thaw on ice, vortex for 30 seconds to mix thoroughly, and briefly centrifuge. If test sample RNA has not already been normalized to 0.1µg/µL concentration, prepare a 0.1µg/µL RNA dilution in nuclease-free water.
11. Add 10 µL of each test sample RNA [0.1µg/µL] into the appropriate BCR-ABL and ABL wells. Carefully dispense the RNA directly into the Master Mix and mix by pipetting the solution up and down three (3) times.
12. Tightly seal the RNA containing wells and the NTC wells and cover the wells for the calibrators containing Master Mixes. The wells are sealed at this point to avoid contamination.

13. Move the plate into the area where calibrators are stored and handled.

14. Vortex the calibrator thoroughly for 30 seconds and briefly centrifuge. It is extremely important to thoroughly mix the calibrators before use.

15. Add 10 µL of each calibrator into the appropriate well. It is important to carefully dispense the calibrator directly into the Master Mix and mix by pipetting the solution up and down three times.

16. Tightly seal all assay wells.

17. Carefully vortex the plate and then centrifuge it briefly to displace any air pockets trapped in the bottom of the wells.

18. Place the plate into the qPCR instrument and program as shown below. The program includes steps for reverse transcription and PCR. Following the reverse transcription step, the PCR amplification must start with an initial heating event at 95°C for two (2) minutes to activate the Taq DNA polymerase.

<table>
<thead>
<tr>
<th>TABLE 7 - RT-qPCR Program</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Cycling Program</strong></td>
</tr>
<tr>
<td>50°C for 15 minutes</td>
</tr>
<tr>
<td>95°C for 2 minutes</td>
</tr>
<tr>
<td>42 cycles: 95°C for 15 seconds; 60°C for 60 seconds</td>
</tr>
</tbody>
</table>

19. Create the plate map on the qPCR instrument. Designate the Calibrator containing wells as standards and enter the appropriate copy number (the copy number of each calibrator is provided in Product Overview Section H). Designate the water controls as NTC and the Control RNAs and test samples as unknowns.

20. Start the cycling program.
Results and Analysis

The acceptability of the run is assessed using the Run Evaluation Criteria (Table 8), which are expected results for the BCR-ABL standard curve and the Control RNAs. Consult the Troubleshooting section of this guide if the results achieved are not within the Run Evaluation Criteria.

Procedure

1. Adjust the threshold and baseline setting on the instrument such that the BCR-ABL standard curve slope and y-intercept comply with the Run Evaluation Criteria. The BCR-ABL standard curve should have a slope of -3.15 to -3.75 and a y-intercept of 35 to 40 for the Applied Biosystems® 7500 Fast or Applied Biosystems® 7500 Fast Dx. The BCR-ABL and ABL Standard Curves should be similar, but may not superimpose. For example, when using the Applied Biosystems® 7500 Fast or Fast Dx instrument, results are best when the Auto Baseline algorithm is selected and the threshold is set at 0.05.

2. Assess the $r^2$ value of the BCR-ABL standard curve. The $r^2$ value should be 0.98 or greater. The 3-copy points of the Calibrator can sometimes deviate from the linear regression line due to subtle pipetting artifacts as some variability is common. A single discordant point may be excluded from the standard curve if it is detrimental to the $r^2$ value.

3. Assess the NTC (water) reactions for false positive amplification. The NTCs should be negative for BCR-ABL and ABL. Detection of BCR-ABL and or ABL at a Ct value of 38 or greater, is an acceptable trace amount.

4. Assess the Negative Control RNA reactions for false positive amplification. The Negative Control RNA reactions should be negative for BCR-ABL, but positive for ABL. Detection of BCR-ABL at a Ct value of 38 or greater, is an acceptable trace amount.

5. Assess the number of ABL copies detected in the High, Low and Negative Control RNA reactions. There should be greater than 10,000 ABL copies in each of the three (3) RNA Controls.

6. The percent BCR-ABL/ABL ratio is calculated by dividing the mean BCR-ABL copy number by the mean ABL copy number and multiplying by 100.

$$\% \text{ BCR-ABL/ABL ratio} = \frac{\text{Mean BCR-ABL Copy Number}}{\text{Mean ABL Copy Number}} \times 100$$

7. Calculate the percent BCR-ABL/ABL ratio of the High and the Low Control RNA reactions. The High Control RNA should have a ratio percent of 6-24% and the Low Control RNA should have a ratio percent of 0.06-0.24%.

8. Calculate the percent BCR-ABL/ABL ratio of the test samples on the International Scale (IS) by multiplying the percent BCR-ABL/ABL ratio by the assay Conversion Factor (CF). A CF value of 0.98 or a laboratory specific CF value harmonized to WHO IS secondary standards can be used to report BCR-ABL/ABL ratio percent values on the International Scale.

$$\% \text{ BCR-ABL/ABL ratio IS} = \% \text{ BCR-ABL/ABL ratio} \times \text{Conversion Factor}$$
### TABLE 8 - Run Evaluation Criteria

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>slope of BCR-ABL Standard Curve</td>
<td>-3.45 ± 0.3</td>
</tr>
<tr>
<td>y- Intercept of BCR-ABL Standard Curve</td>
<td>Ct of 35-40</td>
</tr>
<tr>
<td>r² value of BCR-ABL Standard Curve</td>
<td>&gt; 0.98</td>
</tr>
<tr>
<td>NTC (Water) Control</td>
<td>No amplification</td>
</tr>
<tr>
<td>Low (MR3) Control RNA</td>
<td>&gt; 10,000 ABL copies</td>
</tr>
<tr>
<td>BCR-ABL/ABL ratio of Low Control RNA</td>
<td>0.06% to 0.24%</td>
</tr>
<tr>
<td>High (MR1) Control RNA</td>
<td>&gt; 10,000 ABL copies</td>
</tr>
<tr>
<td>BCR-ABL/ABL ratio of High Control RNA</td>
<td>6% to 24%</td>
</tr>
<tr>
<td>Negative Control RNA</td>
<td>&gt; 10,000 ABL copies, no BCR-ABL copies</td>
</tr>
<tr>
<td>Test Samples</td>
<td>&gt; 10,000 ABL copies</td>
</tr>
</tbody>
</table>
### TABLE 9 - Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
</table>
| Standard Curve slope, y-intercept, and/or $r^2$ value is out of acceptable range. | • Inadequate mixing of the calibrators  
• Poor pipetting technique  
• Non-optimal PCR  
• Threshold and/or baseline not optimal | • Thoroughly vortex the calibrators prior to use  
• Carefully pipette the calibrators into the master mix and mix by pipetting  
• Check instrument for correct cycling conditioning  
• Adjust threshold and/or baseline |
| NTC is positive for BCR-ABL or ABL | • Cross contamination  
• Accidental well inoculation | • Repeat the assay  
• Test reagents for contamination |
| Negative Control RNA is positive for BCR-ABL | • Cross contamination  
• Accidental well inoculation | • Repeat the assay  
• Test reagents for contamination |
| High or Low RNA Control ratios are out of acceptable range | • Incorrect amount of RNA used  
• RNA resuspended in incorrect volume  
• Resuspension of RNA incomplete  
• Poor pipetting technique | • Examine Standard Curves for compliance to run evaluation criteria  
• Review RNA reconstitution protocol and check volume of resuspended RNA  
• Vortex RNA Controls prior to each use  
• Carefully pipette the RNA Controls into the master mix and mix by pipetting |
| Low ABL copies in RNA Controls | • Non-optimal reverse transcription  
• Poor ABL standard curve  
• Insufficient resuspension of RNA Control | • RT-qPCR program must start with 50°C 15 minute step  
• Review handling of calibrators  
• Review RNA Control reconstitution procedure |
| Test Samples have less than 10,000 ABL copies | • Poor RNA quality  
• Inaccurate RNA quantification  
• Reverse transcription step failure | • Check RNA quality and concentration  
• RT-qPCR program must start with 50°C 15 minute step |

Note: If the problem is unresolved by any of the above corrective actions, please contact ClientServices@MolecularMD.com.

### References


Contact Us

If you have any questions or comments for MolecularMD, please feel free to contact us.

Email

ClientServices@MolecularMD.com

Telephone

US Toll Free: 1-877-459-4979
Ph: 1-503-459-4974
Fax: 1-503-459-4976

Address

MolecularMD
1341 SW Custer Drive
Portland, OR 97219
USA