

# Simplexa™ Extraction & Amplification Control Set - RNA (English)

**REF** MOL9200  
Rev. B



For *in vitro* Diagnostic Use

## INTENDED USE

Focus Diagnostics' Simplexa™ Extraction & Amplification Control Set - RNA consists of a RNA template and a labeled primer pair intended to be used as a process control to detect systemic variation that may arise during the extraction and amplification steps of real-time reverse transcription polymerase chain reaction (RT-PCR) assays. The set is not intended to monitor other real-time PCR processes.

## PRINCIPLES OF THE PROCEDURE

The control combines fluorescent probe technology with a real-time RT-PCR amplification and detection system. The probe molecule contains a fluorophore and a quencher. The target sequence is amplified by the primers and binding of the probe element to the amplified DNA fragment results in separation of the fluorophore from the quencher and generation of fluorescent signal.

## MATERIALS PROVIDED

Upon receipt, store all reagents at -10 to -30 °C (do not use a frost-free freezer). After first use, store thawed reagents at 2 to 8 °C up to 30 days. Appropriately stored reagents are stable through the end of the expiration month as indicated on the kit packaging.

**Table 1. Kit Component Table.**

Simplexa™ Extraction & Amplification Control Set - RNA: Part # MOL9200

Kit Component	Part Number	Cap Color	Number in the Kit
Simplexa™ RNA Internal Control	MOL2004	Blue	2
Simplexa™ Extraction & Amplification Control Primer Pair - RNA	MOL9201	Brown	2

**Table 2. Component Description.**

### Simplexa™ RNA Internal Control (250 µL per vial)

<b>REF</b>	<b>MOL2004</b>	<b>CONTROL</b>	<b>IC</b>
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The RNA Internal Control is an encapsulated RNA template.

### Simplexa™ Extraction & Amplification Control Primer Pair – RNA (20 µL per vial)

<b>REF</b>	<b>MOL9201</b>	<b>REAG</b>	<b>A</b>
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The Extraction & Amplification Control Primer Pair - RNA consists of a Quasar® 670 labeled integrated probe and a forward primer and a reverse primer pair specific for the RNA Internal Control template.

## SHELF LIFE AND HANDLING

1. Store reagents at -10 to -30 °C (do not use a frost-free freezer).
2. Do not use kits or reagents beyond their expiration dates.
3. Allow reagents to thaw at room temperature (approximate range 18 to 25 °C) before use.
4. Once thawed, store the unused reagent at 2 to 8 °C for no more than 30 days.
5. Do not refreeze.
6. Store materials in the dark and protect from light.
7. When properly stored, kit reagents are stable through the end of the month indicated on the expiration date on the kit packaging.

## WARNINGS AND PRECAUTIONS

1. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
2. Do not pipette by mouth.
3. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
4. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
5. Workflow in the laboratory should proceed in a uni-directional manner, beginning in the Pre-Amplification Area(s) and moving to the Amplification/Detection Area. Each step should be performed in a dedicated area. Supplies and equipment used for reagent preparation should not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target nucleic acid. Post-amplification supplies and equipment should remain in the Amplification/Detection Area at all times. Disposable gloves must be worn in each area and must be changed before leaving that area.
6. Contamination of patient specimens or reagents can produce erroneous results. Use aseptic techniques.
7. Pipette and handle reagents carefully to avoid mixing of samples from adjacent wells.
8. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
9. Do not interchange the reagent tube caps. This may cause contamination and compromise the test results.
10. Store or discard unused samples according to your laboratory standard operating procedures.
11. Listed volumes of Simplexa Extraction & Amplification RNA Internal Control and Primer Pair are suggested volumes and may be modified to work with the parameters of your real-time RT-PCR assay.

**INSTRUCTIONS FOR USE****I. SPECIMEN PREPARATION AND HANDLING**

*Performed in area dedicated for specimen extraction*

*Use RNase/DNase-free tubes only*

1. Add Extraction & Amplification RNA Internal Control to each clinical sample prior to extraction. (Recommended volume is 5µL)

**II. RT-PCR REACTION REAGENT PREPARATION**

*Performed in area dedicated for PCR reagent preparation.*

*Use RNase/DNase-free tubes only*

1. Prepare real-time RT-PCR reagents and set up according to your validated real-time PCR procedure.
2. Add Simplexa Extraction & Amplification Control Primer Pair - RNA to the assay mix. (Recommended volume is 1/50 of the total assay volume).

**III. RT-PCR REACTION**

*Performed in area dedicated for PCR reactions.*

1. Run real-time RT-PCR assay.
2. After the completion of real-time RT-PCR run, view the Simplexa Extraction & Amplification RNA Internal Control amplification curve by selecting a detector/channel that can detect fluorescence from a Quasar® 670 dye\*. Set appropriate threshold and determine C<sub>T</sub> values.  
**\*Note: Quasar® 670 Dye has 644 nm absorption maxima and 670 nm emission maxima.**

**QUALITY CONTROL**

Simplexa Extraction & Amplification RNA Internal Control is an unassayed control. Control ranges may vary based on the combination of extraction methods and RT-PCR protocols used. A specific range should be determined for each desired extraction and amplification method. Each laboratory should establish their own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice.

**LIMITATIONS**

1. The Extraction & Amplification Control Set - RNA can be used to detect systemic variation that may arise during the extraction and amplification process associated with real-time RT-PCR assays. The set is not intended to monitor other real-time RT-PCR processes.
2. The dye labeled primer utilizes Quasar® 670 dye. The real-time RT-PCR assay should be run on a system with the ability to detect from 644 nm to 670 nm.

**AUTHORIZED REPRESENTATIVE**

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**ORDERING INFORMATION**

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