Quick-Start Protocol April 2015

QuantiNova™ Internal Control RNA and Assay

The QuantiNova Internal Control RNA (QN IC RNA) in the QuantiNova Reverse Transcription Kit (cat. nos. 205410, 205411, 205413), QuantiNova Probe RT-PCR Kit (cat. nos. 208352, 208354, 208356) and QuantiNova SYBR® Green RT-PCR Kit (cat. nos. 208152, 208154, 208156) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- Kit Handbooks: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

Notes before starting

- The QN IC RNA is a synthetic RNA that can be optionally used to monitor successful reverse transcription. The QN IC RNA is intended to report instrument or chemistry failures, errors in assay setup and the presence of inhibitors. Inhibitors such as phenol, ethanol, sodium dodecyl sulfate (SDS) or ethylene diaminetetraacetic acid (EDTA) may remain from the lysis and purification steps of the RNA isolation procedure.
- The primer and probe sequences for the detection of the QN IC RNA have been bioinformatically validated for non-homology against hundreds of eukaryotic and prokaryotic organisms. Additionally, they have been experimentally tested against a multitude of human, mouse and rat RNA samples from multiple tissues and cell lines.
- The QN IC RNA is detected as a 200 bp amplicon. For SYBR Green-based detection, use the Ctrl_QNIC_1_SG QuantiTect® Primer Assay (cat. no. QT02589307) and the QuantiNova SYBR Green PCR and RT-PCR Kits. For probe-based detection, use the QuantiNova IC Probe Assay (cat. no. 205813). The

QN IC RNA can be detected using the VIC®/HEX dye channel of your real-time PCR instrument and the QuantiNova Probe PCR and RT-PCR Kits.

- The QN IC RNA (optional) is added to the experimental RNA sample. An additional no-template RNA control sample, which only contains the QN IC RNA, should also be set up. C_q shifts >2 between the template RNA+QN IC RNA compared to the QN IC RNA only samples, and between the different template RNA containing samples indicate inhibition of the RT-PCR.
- For the QuantiNova Reverse Transcription Kit, the QN IC RNA is used undiluted. Before use with the QuantiNova Probe RT-PCR Kit and the QuantiNova SYBR Green RT-PCR Kit, dilute the QN IC RNA 1:10 by adding 180 µl (20 µl tube) or 900 µl (100 µl tube) of RNase-free water to the RNA. Mix thoroughly by vortexing.

1. Mix the QuantiNova Internal Control RNA thoroughly by vortexing, and dispense 1 µl into each sample as described in the quick-start protocols for the QuantiNova Reverse Transcription Kit (undiluted IC) and the QuantiNova SYBR Green or Probe RT-PCR Kits (pre-diluted IC).

2a. For analysis with the QuantiNova Probe RT-PCR Kit, add the appropriate volume of 10x QuantiNova IC Probe Assay to the sample. Signal detection is performed on the filter/channel for HEX/VIC of your real-time PCR instrument. For analysis with the QuantiNova SYBR Green RT-PCR Kit, use the appropriate volume of 10x QuantiTect Primer Assay in the reaction.

Note: QuantiNova IC Assays, for the detection of QN IC RNA, need to be ordered separately as the QuantiNova IC Probe Assay (cat. no. 205813) or the Ctrl_QNIC_1_SG QuantiTect Primer Assay (cat. no. QT02589307, for SYBR Green detection) at GeneGlobe (**www.qiagen.com/geneglobe**).

2b. If using the QuantiNova Reverse Transcription Kit, cDNA should be diluted (1:10–1:100) and an aliquot of the reaction should be used for subsequent amplification with the QuantiNova SYBR Green or Probe PCR Kits.

For probe-based detection, use the QuantiNova IC Probe Assay and detection in the VIC/HEX dye channel of your real-time PCR instrument. For detection with the QuantiNova SYBR Green PCR Kit, use the Ctrl_QNIC_1_SG QuantiTect Primer Assay.

Data analysis and interpretation of results

1. After amplification, perform data analysis as recommended for your real-time PCR instrument.
2. Compare C_q values between the QN IC RNA only and samples containing QN IC RNA plus template RNA.

   Consistent C_q values indicate successful RT-PCR and reliable results. A C_q difference >2 is likely to indicate inhibition or sample failure.
3. If a shifted C_q of >2 appears, indicating inhibition or failure of a specific sample, we recommend the following:
   a. Check equipment for accurate performance and repeat sample/experiment to rule out pipetting or handling errors.
   b. Dilute the affected template RNA using RNase-free water before repeating the experiment. This dilutes inhibitors present in the sample.
   c. Consider repeating the RNA extraction and avoid contamination or carry-over of inhibitors (e.g., use an appropriate RNeasy® Kit).

   Alternatively, the RNeasy MinElute Cleanup Kit (cat. no. 74204) can be used to remove potential inhibitors and concentrate the RNA template.

Expected C_q value for successful RT-PCR (e.g., no inhibition of QN IC RNA)

The C_q value for the QN IC RNA in 2-step RT-PCR using the QuantiNova Reverse Transcription Kit will vary depending on various parameters, such as:

- Detection format (SYBR Green or probe)
- Dilution factor of the cDNA after reverse transcription
- Volume of template cDNA added to the PCR

- Type of real-time PCR instrument
- C_q determination (threshold setting, Auto-C_q determination)

An example of typical results for the QN IC RNA:

- Dilution factor: 10
- Volume of cDNA added to PCR: 2µl
- Real-time instrument: Rotor-Gene® Q
- C_q determination: manual threshold setting
- C_q value with QuantiNova SYBR Green PCR Kit: 21
- C_q value with QuantiNova Probe PCR Kit: 27

This example illustrates that C_q values for the QN IC RNA in 2-step RT-PCR, using the QuantiNova Reverse Transcription Kit, will not be identical under all circumstances. However, the C_q values should be consistent under identical conditions; therefore, the QN IC RNA can be used to monitor successful reverse transcription and RT-PCR. A C_q difference >2 is likely to indicate sample inhibition or failure.

Typical results for the QN IC RNA in 1-step RT-PCR:

The C_q value for the QN IC RNA in the QuantiNova Probe RT-PCR Kit depends on the real-time PCR instrument used and can be expected within a C_q range of 23–25.

The C_q value for the QN IC RNA in the QuantiNova SYBR Green RT-PCR Kit depends on the real-time PCR instrument used and can be expected within a C_q range of 17–19.