September 2026

Quick-Start Protocol

dPCR RNA-Plex Assays (Part 2: cDNA Templates)

This protocol describes the quantification of human, mouse, or rat mRNA and lncRNA from cDNA templates using dPCR RNA-Plex Assays (cat. nos. 250336 and 250337) on the QIAcuity® Digital PCR System. The assays support singleplex and multiplex detection in six channels with the QIAcuity High Multiplex Probe PCR Kit (cat. nos. 250133, 250134) or in five channels with the QIAcuity Probe PCR Kit (cat. nos. 250101, 250102, 250103). Visit **www.qiagen.com** for product details.

Assays are supplied as dried-down mixes. Store protected from light at –30°C to –15°C upon receipt; under these conditions, the assays are stable until the expiration date on the vial.

Further information

- *dPCR RNA-Plex Assay Handbook*: **www.qiagen.com/HB-3878**
- *QIAcuity User Manual*: **www.qiagen.com/HB-2717-012**
- *QIAcuity Application Guide*: **www.qiagen.com/HB-2839**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Important points before starting

- Resuspend the dried-down assay mixes before use. Dissolved dPCR assay mix can be stored at 2–8°C for short-term storage (up to 3 weeks). Avoid repeated freeze–thaw cycles. If possible, store the assay in aliquots.
- We recommend using multiple reference target assays depending on the expression level of the genes of interest. For more information on reference target assay selection, see the *dPCR RNA-Plex Assay Handbook*.
- Make sure that all pipettes and instruments have been checked and calibrated according to the manufacturer's recommendations. During pipetting, make sure that no bubbles are introduced into the wells of QIAcuity Nanoplate.
- QIAcuity High Multiplex Kit and QIAcuity High Multiplex Probe PCR Kit require QIAcuity Software Suite version 3.0 or later.
- QuantiTect Rev. Transcription Kit (cat. nos. 205311, 250313, 250314) is recommended for reverse transcription.

Assay resuspension

For ease of use, we recommend preparing a 20× or 40× concentrated assay mix. For higher-order multiplex reactions, 40× assay mixes are advisable because they leave more room for template in the reaction. To achieve the respective concentration, add the amounts specified in Table 1 to the tube using 1× low TE buffer (10 mM Tris·Cl, pH 8.0, 0.1 mM EDTA). Mix thoroughly and let the solution stand for 20 min at ambient temperature to ensure that the assay mix fully dissolves. We do not recommend resuspension in water.

Table 1. Assay resuspension guidelines to achieve a 20x or 40x assay mix

	Amount of 1x low TE (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) to be added	
Assay product (cat. no.)	**20x assay mix**	**40x assay mix**
dPCR RNA-Plex Assay 250 rxn (250336)	165 µL	82.5 µL
dPCR RNA-Plex Assay 750 rxn (250337)	495 µL	247.5 µL

Reaction setup

1. Thaw the selected master mix, template cDNA, dPCR RNA-Plex Assays, and RNase-free water. Mix each solution by briefly vortexing, followed by brief centrifugation.
2. Prepare the reaction mix containing all components except the template according to the master mix and application used:

   QIAcuity High Multiplex Probe PCR Kit: Use Table 2.

   QIAcuity Probe PCR Kit: Use Table 3.

   QIAcuity OneStep High Multiplex PCR Kit: Consult Part 1 of the QSP for RNA-Plex Assays with RNA Templates (qiagen.com/HB-3871).

   Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument.
3. Vortex the reaction mix.

Table 2. Reaction setup for detecting up to 6 or 12 cDNA targets with QIAcuity High Multiplex Probe PCR Kit

	Volume/reaction		
Component	**Nanoplate 8.5k (24- and 96-well)**	**Nanoplate 26k (8- and 24-well)**	**Final concentration**
4× QIAcuity High Multiplex Probe PCR Master Mix	3 µL	10 µL	1×
20× Primer–Probe Mix 1–6 (one target per channel)*	0.6 µL (each)	2 µL (each)	1×†
20× Primer–Probe Mix 7–12 (amplitude-based multiplexing)*	Variable	Variable	Variable†
RNase-free water	Variable	Variable	–
Template cDNA (added at step 4)‡	Variable	Variable	–
Total reaction volume	**12 µL**	**40 µL**	

* For higher-order multiplexing, 40× assay mixes may be used to allow more sample volume.

† Amplitude-based multiplexing requires variable assay concentrations; see the *QIAcuity High Multiplex Probe PCR Kit Handbook*.

‡ Appropriate template amount depends on various parameters. For details, see the *QIAcuity Application Guide*.

Table 3. Reaction setup for detecting up to 5 cDNA targets with QIAcuity Probe PCR Kit

Component	Volume/reaction		Final concentration
	Nanoplate 8.5k (24- and 96-well)	Nanoplate 26k (8- and 24-well)	
4× QIAcuity High Multiplex Probe PCR Master Mix	3 µL	10 µL	1×
20× Primer–Probe Mix 1–5	0.6 µL (each)	2 µL (each)	1×
RNase-free water	Variable	Variable	–
Template cDNA (added at step 4)*	Variable	Variable	–
Total reaction volume	**12 µL**	**40 µL**	

* Appropriate template amount depends on various parameters.

4. Dispense the appropriate volumes of the reaction mix, containing all components except the template, into the wells of a standard PCR plate. Then, add cDNA into each well containing the reaction mix.

   Note: The appropriate amounts of reaction mix and cDNA depend on various parameters. For details, see the *QIAcuity Application Guide: QIAcuity User Manual Extension*.
5. Transfer the contents of each well of the standard PCR plate to the wells of a 26k or 8.5k Nanoplate.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in QIAcuity Nanoplate Kits. For the exact sealing procedure, see the *QIAcuity User Manual*.

Thermal cycling and imaging conditions

1. Set the cycling conditions under the dPCR parameters in the QIAcuity Software Suite or on the QIAcuity instrument according to Table 4.

Table 4. Cycling conditions for reactions on cDNA templates

Step	Time	Temperature (°C)
Initial denaturation	2 min	95
2-step cycling (40 cycles)		
Denaturation	15 s	95
Combined annealing/extension	1 min	60

2. Activate the required channels in **Imaging** under the dPCR parameters in QIAcuity Software Suite or on the QIAcuity instrument.
3. Place the nanoplate in the QIAcuity instrument and start the dPCR program.
4. Image the plate using the imaging settings in Table 5.

Table 5. Imaging settings for the dPCR RNA-Plex Assays

Channel	Exposure (ms)	Gain
Green	500	8
Yellow	500	8
Orange	280	6
Red	250	10
Crimson	500	10
Far red	1200	12

Note: Imaging settings may need to be adjusted depending on the assay. Always start with the recommended settings.

Document Revision History

Date	Description
09/2026	Initial release

Scan the QR code for *QIAcuity User Manual*. For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.
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