March 2024

Quick-Start Protocol

Q-Solution® Kit

For the vast majority of applications on the QIAcuity® platform, users can simply combine their template and assays with any of the uniquely formulated QIAcuity master mixes to obtain excellent results. However, for certain challenging instances, adjusting the QIAcuity reaction chemistry may be necessary to ensure best performance. For these cases, we have developed the Q-Solution Kit, which contains a 5x Q-Solution and a standalone solution of 25 mM magnesium chloride ($MgCl_2$).

Q-Solution is an innovative and versatile PCR additive that works in part by lowering the melting temperature of DNA. Adjusting template melting properties can be useful when interrogating DNA with high-GC content or with complex secondary structures, like Adeno-associated virus (AAV) genomes. Q-Solution has additional properties that can be used to mitigate the carryover of PCR inhibitors, like ethanol and heparin.

In contrast to Q-Solution, the $MgCl_2$ supplied with the kit stabilizes double stranded DNA structures, including those formed between primers and template. If weak primer or probe binding is suspected to be underlying poor PCR performance, $MgCl_2$ may be used to boost primer binding affinity. Magnesium chloride is also an essential cofactor for DNA polymerase function. Carryover of magnesium chelators like EDTA into a PCR reaction may cause total PCR failure. This can be mitigated by adding additional $MgCl_2$ to the reaction.

Q-Solution and $MgCl_2$ can be used alone or in combination. Particularly high concentrations of Q-Solution (e.g., >1x) can lower the available concentration of Magnesium in the PCR reaction. Therefore, in applications requiring high levels of Q-Solution, adding $MgCl_2$ to the reaction will be necessary for best performance. This Quick-Start Protocol will highlight a few

examples of how the Q-Solution Kit can be used in QIAcuity workflows. However, the utility of the Q-Solution Kit is not limited to the use cases presented here. Furthermore, the concentrations of Q-Solution and $MgCl_2$ used in the examples shown here may not work for all use cases.

Further information

- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- *QIAcuity User Manual Extension*: **www.qiagen.com/HB-2839**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

The Q-Solution Kit should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. Unless otherwise indicated on the label, the components are stable for 36 months without showing any reduction in performance under these conditions.

On the QIAcuity, up to 830 (12 µL) dPCR reactions or 250 (40 µL) dPCR reactions can be performed with one kit, when using 1x Q-Solution and 3mM $MgCl_2$.

Notes before starting

- Refer to the Quick Start Protocol of the according Kits for detailed procedures:
  - QIAcuity Probe PCR Kit: **www.qiagen.com/HB-2792**
  - QIAcuity OneStep Advanced Probe Kit: **www.qiagen.com/HB-3048**
  - QIAcuity EG PCR Kit: **www.qiagen.com/HB-2791**
  - QIAcuity UCP Probe PCR Kit: **www.qiagen.com/HB-3100**

- Thaw the Q-Solution and $MgCl_2$. Vigorously mix the individual solutions. Centrifuge briefly to collect liquids at the bottom of the tubes

Procedure

As a universal approach Q-Solution can be added with 1x concentration in the dPCR reaction. If necessary the concentration range can be titrated from 0.25x to 2x.

High concentrations of Q-Solution (e.g., >1x) can lower the available concentration of Magnesium in the PCR reaction. Therefore, in applications requiring high levels of Q-Solution, adding $MgCl_2$ to the reaction may be necessary for best performance starting with 3.4mM. If necessary, the concentration range can be titrated from 0mM to 6.7mM.

For detailed examples and further guidance, please use the application note. For cases not mentioned in the QSP or Application note, please proceed as described above and use Q-Solution in the first step. If you still experience unsatisfactory performance adjust the concentration and/or use and adjust $MgCl_2$ concentration.

Table 1. Preparing the QIAcuity Probe PCR reaction mix

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well and 96-well)**	**Nanoplate 26k (8-well and 24-well)**	**Final concentration**
4x Probe PCR Master Mix	3 µL	10 µL	1x
10x primer-probe mix 1*	1.2 µL†	4 µL†	0.8 µM forward primer 0.8 µM reverse primer 0.4 µM probe
10x primer-probe mix 2, 3, 4, 5* (for multiplex)	1.2 µL†	4 µL†	0.8 µM forward primer 0.8 µM reverse primer 0.4 µM probe
Restriction Enzyme (optional)	Up to 1 µL	Up to 1 µL	0.025–0.25 U/µL
5x Q-Solution	2.4 µL‡	8 µL‡	1x
25mM $MgCl_2$	Variable	Variable	0mM§
Rnase-free water	Variable	Variable	
Template DNA or cDNA	Variable¶	Variable¶	
Total reaction volume	**12 µL**	**40 µL**	

* For respective dye recommendation for the probe and available channels on the QIAcuity, please see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension: Application Guide*.

† Volume might vary, depending on the concentration of the primer/probe mix used.

‡ As a universal approach, the Q-Solution can be added with 1x concentration in the dPCR reaction. If necessary, the concentration range can be titrated from 0.25x to 2x.

§ High concentrations of Q-Solution (e.g. >1x) can lower the available concentration of Magnesium in the PCR reaction. Therefore, in applications requiring high levels of Q-Solution, adding $MgCl_2$ to the reaction may be necessary for best performance starting with 3.4mM. If necessary the concentration range can be titrated from 0mM to 6.7mM.

¶ Appropriate template amount depends on various parameters. Please see the *QIAcuity User Manual Extension: Application Guide* for details.

Table 2. Preparing the QIAcuity OneStep Advanced Probe PCR reaction mix

Component	Volume/reaction		
	Nanoplate 8.5k (24-well and 96-well)	Nanoplate 26k (8-well and 24-well)	Final concentration
4x OneStep Advanced Probe Master Mix	3 µL	10 µL	1x
100x OneStep Advanced RT Mix (Reverse Transcription)	0.12 µL	0.4 µL	1x
20x primer-probe mix 1*	0.6 µL	2 µL	0.4 µM forward primer 0.4 µM reverse primer 0.2 µM probe
20x primer-probe mix 2, 3, 4, 5* (for multiplex)	0.6 µL (each)	2 µL (each)	0.4 µM forward primer 0.4 µM reverse primer 0.2 µM probe
Enhancer GC† (optional)	1.5 µL	5 µL	–
5x Q-Solution	2.4 µL‡	8 µL‡	1x
25mM MgCl2	Variable	Variable	0mM§
Rnase-free water	Variable	Variable	
Template RNA¶	Variable	Variable	
Total reaction volume	**12 µL**	**40 µL**	

* For respective dye recommendation, see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension*.

† Enhancer GC is recommended for use with all Applied Biosystems TaqMan® assays, amplicons >150 bp in length, GC-rich amplicons, and RNA targets containing challenging secondary structures.

‡ As a universal approach Q-Solution can be added with 1x concentration in the dPCR reaction. If necessary, the concentration range can be titrated from 0.25x to 2x.

§ High concentrations of Q-Solution (e.g. >1x) can lower the available concentration of Magnesium in the PCR reaction. Therefore, in applications requiring high levels of Q-Solution, adding $MgCl_2$ to the reaction may be necessary for best performance starting with 3.4mM. If necessary, the concentration range can be titrated from 0mM to 6.7mM.

¶ Appropriate template amount depends on various parameters.

Table 3. Preparing the QIAcuity EvaGreen® PCR reaction mix

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well and 96-well)**	**Nanoplate 26k (8-well and 24-well)**	**Final concentration**
3x EvaGreen PCR Master Mix (FAM channel)	4 µL	13.3 µL	1x
10x primer mix	1.2 µL*	4 µL*	0.4 µM forward primer 0.4 µM reverse primer
Restriction Enzyme (optional)	Up to 1 µL	Up to 1 µL	0.025–0.25 U/µL
5x Q-Solution	2.4 µL†	8 µL†	1x
25mM $MgCl_2$	Variable	Variable	0mM‡
Rnase-free water	Variable	Variable	
Template DNA or cDNA	Variable§	Variable§	
Total reaction volume	**12 µL**	**40 µL**	

* Volume might vary, depending on concentration of the primer/probe mix used.

† As a universal approach, the Q-Solution can be added with 1x concentration in the dPCR reaction. If necessary, the concentration range can be titrated from 0.25x to 2x.

‡ High concentrations of Q-Solution (e.g. >1x) can lower the available concentration of Magnesium in the PCR reaction. Therefore, in applications requiring high levels of Q-Solution, adding $MgCl_2$ to the reaction may be necessary for best performance starting with 3.4mM. If necessary the concentration range can be titrated from 0mM to 6.7mM.

§ Appropriate template amount depends on various parameters. Please see the *QIAcuity User Manual Extension: Application Guide* for details.

Table 4. Preparing the QIAcuity UCP Probe PCR reaction mix

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well and 96-well)**	**Nanoplate 26k (8-well and 24-well)**	**Final concentration**
4x UCP Probe Master Mix	3 µL	10 µL	1x
20x primer-probe mix 1*	0.6 µL†	2 µL†	0.6 µM forward primer 0.6 µM reverse primer 0.2 µM probe
20x primer-probe mix 2, 3, 4, 5* (for multiplex)	0.6 µL†	2 µL†	0.6 µM forward primer 0.6 µM reverse primer 0.2 µM probe
Restriction Enzyme (optional)	Up to 1 µL	Up to 1 µL	0.025–0.25 U/µL
5x Q-Solution	2.4 µL‡	8 µL‡	1x
25mM $MgCl_2$	Variable	Variable	0mM§
UCP PCR water	Variable	Variable	
Template DNA or cDNA	Variable¶	Variable¶	
Total reaction volume	**12 µL**	**40 µL**	

* For respective dye recommendation for the probe and available channels on the QIAcuity, please see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension: Application Guide*.

† Volume might vary, depending on the concentration of the primer/probe mix used. For TaqMan® probes, we suggest a final concentration of 0.8µM for each primer and 0.4 µM for each probe.

‡ As a universal approach, the Q-Solution can be added with 1x concentration in the dPCR reaction. If necessary the concentration range can be titrated from 0.25x to 2x.

§ High concentrations of Q-Solution (e.g. >1x) can lower the available concentration of Magnesium in the PCR reaction. Therefore, in applications requiring high levels of Q-Solution, adding $MgCl_2$ to the reaction may be necessary for best performance starting with 3.4mM. If necessary the concentration range can be titrated from 0mM to 6.7mM.

¶ Appropriate template amount depends on various parameters. Please see the *QIAcuity User Manual Extension: Application Guide* for details.

Document Revision History

Date	Changes
March 2024	Initial release.

Scan QR code for the *QIAcuity User Manual*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.