July 2024

Product Sheet

QIAcuityDx® Universal MasterMix Kit

Version 1

IVD

For In Vitro Diagnostic Use

For Laboratory Use

CE

REF 260101, 260102

QIAGEN, GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

R1 MAT 1134829

Sample to Insight

Table of Contents

Kit Contents

Cat. no. Kit	260101 1 mL	260102 5 mL
QIAcuityDx Universal MasterMix	1 x 1180 µL	5 x 1180 µL
$MgCl_2$, 200mM	1 x 1000 µL	2 x 1000 µL
RNase-free water	2 x 1.9 mL	5 x 1.9 mL

Shipping and Storage

The QIAcuityDx Universal MasterMix Kit is shipped on dry ice. It should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. If any component of the QIAcuityDx Universal MasterMix Kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note, or the reagents, please contact QIAGEN Technical Services or local distributors (visit **www.qiagen.com**).

When stored correctly, the QIAcuityDx Universal MasterMix Kit is stable until the expiration date printed on the label.

Do not use if stored outside the specifications, if the packaging has been damaged, or if other signs of deterioration or malfunction are visible.

In-use stability

Once opened, reagents can be stored in their original packaging at –30 to –15°C until the stated expiry date shown on the packaging. Repeated thawing and freezing should be avoided. Do not exceed a maximum of five freeze–thaw cycles.

The reagents must be fully thawed at room temperature (15–25°C) for maximum of 30 minutes before use.

Intended Use

The QIAcuityDx Universal MasterMix Kit is a ready-to-use general purpose dPCR master mix reagent set for use with the QIAcuityDx Four instrument in conjunction with associated assay specific reagents as part of validated diagnostic test procedures.

The QIAcuityDx Universal MasterMix Kit is not an automated device and is intended for laboratory use by trained personnel.

The QIAcuityDx Universal MasterMix Kit is intended for *in vitro* diagnostic use.

It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the QIAGEN performance studies.

Active ingredients

Reagent	Name	Active Ingredient	Concentration (% w/w)
Master mix	QIAcuityDx Universal MasterMix	QuantiNova® DNA Polymerase (5.6 U/µL)	12%
		dNTP Mix (10 mM each)	10%
Magnesium Chloride	$MgCl_2$, 200mM	None	–
Water	RNase-free water	None	–

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

CE	This product fulfils the requirements of the European Regulation (EU) 2017/746 for in vitro diagnostic medical devices (IVDR).
IVD	*In vitro* diagnostic medical device
REF	Catalog number
MAT	Material number
LOT	Lot number
GTIN	Global Trade Item Number
UDI	Unique Device Identifier
CONT	Contains
COMP	Component
NUM	Number
	Date of manufacture
Rn	R is for revision of the Product Sheet and n is the revision number
Vn	V is for version of the Product Sheet and n is the version number
	Use-by date

Temperature limitations

Legal manufacturer

Consult instructions for use

Contains reagents sufficient for <N> reactions

Protect from light

Warning

Health Hazard

Safety Information

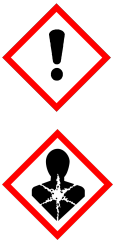
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.

Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

QIAcuityDx Universal MasterMix Kit contains QuantiNova DNA Polymerase, which is produced by a process of bacterial fermentation. The enzyme is purified from the microbes at the end of processing to remove any residual source of potentially infectious material.

Universal MasterMix



Contains: 2-methylisothiazol-3(2H)-one; 1,2,4-triazole. May cause an allergic skin reaction. May damage fertility or the unborn child. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves/ protective clothing/ eye protection/ face protection. If exposed or concerned: Get medical advice/ attention. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Emergency information

CHEMTREC

USA & Canada: 1-800-424-9300

Outside USA & Canada: +1 703-527-3887

Description and Principle

The QIAcuityDx Universal MasterMix Kit comprises of a ready-to-use dPCR master mix containing reaction chemistry in PCR Buffer and proprietary reference dye, and separate tubes of 200mM Magnesium Chloride ($MgCl_2$) 100% w/w and RNase-free water 100% w/w.

A full list of materials to be used with the QIAcuityDx Universal MasterMix Kit can be found in the *QIAcuityDx System User Manual*.

This protocol is optimized for the quantification of DNA or cDNA targets using the QIAcuityDx Universal MasterMix Kit with TaqMan® probes in a singleplex or multiplex reaction using the QIAcuityDx System.

Notes before starting

- A fluorescent dye is provided as a component of the QIAcuityDx Universal MasterMix Kit for reliable detection of proper partition filling in the QIAcuityDx compatible nanoplates.
- For the highest efficiency dPCR assay using TaqMan probes, amplicons should ideally be 60–150 bp in length. Similar to qPCR, longer amplicons may also be used, however, assay performance may be impaired.
- Before performing multiplex analyses, choose suitable combinations of reporter dyes and quenchers that are compatible with multiplex analyses using the detection optics of the QIAcuityDx Four instrument (see Table 1).

  Important: An integrated cross-talk correction is applied to images generated by the QIAcuityDx Four instrument. This correction is to minimize the effects of spectral overlap between neighboring optical channels and fluorophores. Use of non-supported dyes may result in sub-optimal cross-talk correction.

Table 1. Optical channels and supported fluorophores for the QIAcuityDx Four instrument

Channel	Excitation (nm)	Emission (nm)	Supported fluorophores
Green	463–503	518–548	FAM™
Yellow	514–535	550–564	HEX™
Orange	543–565	580–606	TAMRA™
Red	570–596	611–653	ROX™
Crimson	590–640	654–692	Cy5®

- Non-fluorescent quenchers should be used with each probe. Double quenched probes may be utilized to improve signal-to-noise ratios in certain assays.

- It is recommended to start assay development with the cycling conditions and primer concentrations specified in this protocol. The PCR cycling conditions must start with an initial incubation step of 2 minutes at 95°C to activate the QuantiNova DNA Polymerase in the QIAcuityDx Universal MasterMix Kit.
- For ease of use, we recommend preparing a 10x or higher, concentration of primer-probe mix containing target-specific primers and probe for each of your targets. A 10x primer-probe mix consists of 1–8 µM forward primer, 1–8 µM reverse primer, and 0.5–4 µM probe in TE buffer with low EDTA (0.1 mM).
- DNA template with a >30 kb average length may need to be fragmented by restriction digestion before partitioning. Enzymatic fragmentation of larger DNA ensures even distribution of template throughout the QIAcuityDx compatible nanoplate, which in turn ensures accurate and precise quantification. Restriction digestion is not required for highly fragmented DNA (e.g. FFPE DNA or circulating DNA) or cDNA. Care should be taken to use enzymes that will not cut within the amplified sequence, therefore restriction enzymes are recommended.
- Sample input amounts should be based upon the nanoplate partition numbers, with an upper limit of 5 copies per partition when using TaqMan probe based detection (Table 2). The ideal range of copies/partition is between 0.5–3. If the copy number cannot be determined prior to the start of the experiment, it is recommended to perform an initial titration experiment to determine the optimal sample input amount.

Table 2. Maximum copy number per reaction per plate type

Plate type	Number of partitions	Upper limit of copies per reaction	Analyzed volume (µL)	Total reaction volume (µL)	Max. copy number per analyzed volume	Estimated max. copy number per reaction
8.5k Nanoplates	8500	5	2.9	13	42,500	170,000
26k Nanoplates	26,000	5	24.0	42	130,000	217,000

Procedure

1. Thaw the QIAcuityDx Universal MasterMix, Magnesium Chloride, template DNA or cDNA, primer-probe mix and RNase-free water at room temperature for a maximum of 30 minutes.
2. Mix each of the solutions by vortexing at full speed for 3–5 seconds. The tubes should be centrifuged briefly after mixing to collect the liquids at the bottom of the tubes.
3. Prepare an assay master mix for the number of reactions needed according to Table 3, minus the template / No Template Control (NTC). It is not necessary to keep samples on ice during reaction set-up or subsequent steps.

Table 3. Recommended assay master mix set-up

Component	Volume/well (24/96-well, 8.5k Nanoplates)	Volume/well (24-well, 26k Nanoplates)	Final concentration
QIAcuityDx Universal MasterMix	3.3 µL	11 µL	1x
$MgCl_2$, 200 mM	0.41 µL*	1.38 µL*	6.28 mM*
10x primer-probe mix (per assay)†	1.32 µL†	4.4 µL†	0.1–0.8 µM forward primer 0.1–0.8 µM reverse primer 0.05–0.4 µM probe
Restriction enzyme (optional)	Up to 1 µL	Up to 1 µL	0.025–0.25 U/µL
RNase-free water	Variable	Variable	
Template DNA or cDNA (added at step 5)	Variable‡	Variable‡	
Total	**13.2 µL**	**44 µL**	

*Recommended starting concentration, volume may vary depending on optimisation.

†Volume may vary, depending on the concentration of the primer-probe mix used and final target concentration.

‡Appropriate template amounts depends on various parameters, see notes before starting.

4. Mix the master mix by vortexing at full speed for 3–5 seconds. Centrifuge briefly.

5. Dispense appropriate volumes of the assay master mix, which contains all components except the template / No Template Control (NTC) into wells of a standard PCR plate or lo-bind tubes. Then, add template DNA/NTC into each well/tube at the volume appropriate to your assay (see Notes before starting).

   Note: For 2-step RT-PCR, the volume of cDNA added (from the undiluted reverse-transcription reaction) should not exceed 15% of the final PCR volume.

6. Mix the submix (assay master mix and template) either in a PCR plate by pipetting up and down 10 times in the well, or if mixing in a tube, by vortexing at full speed for 3–5 seconds. Centrifuge the plate/tube briefly to collect the liquid at the bottom of the well/tube.
7. Transfer the contents of each well/tube immediately to the wells of the nanoplate.

   Note: Ensure no air bubbles are created during the transfer to the nanoplate by pipetting to the first stop. Ensure to pipette the mix into the input well and not the output well. To avoid damaging the optical surface and to reduce dust that will interfere with the imaging and analysis of results, we recommend to place the nanoplate into a nanoplate tray before pipetting the reaction mix into the nanoplate. The nanoplate tray should be pre-cleaned with a lint-free tissue before use.

8. Seal the nanoplates properly using the nanoplate seal provided in the plate kits.

   Note: For exact sealing procedure, please see the *QIAcuityDx System User Manual*.

9. If a restriction enzyme for DNA digestion has been included in the reaction, leave the plate for 10 minutes at room temperature.
10. Program the cycler of the QIAcuityDx Four instrument according to Table 4.

Table 4. Recommended dPCR cycling conditions

Step	Time	Temperature (°C)	No. of cycles
PCR initial heat activation	2 min	95	1
Denaturation	15 s	95	40*
Combined annealing/extension*	30s*	60	

*Temperature/time/number of cycles might vary depending on assay type

11. Place the nanoplate into the QIAcuityDx Four instrument and start the dPCR program according to the *QIAcuityDx System User Manual*.

Disposal

Dispose used and unused product in compliance with local and national regulations. Follow the recommendations in the Safety Data Sheet (SDS).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAcuityDx Universal MasterMix Kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

The QIAcuityDx Universal MasterMix Kit's performance has been established with the applicable downstream QIAGEN assays. Please consult the respective instructions for use of the respective QIAGEN downstream application for detailed instructions on handling of this product within the corresponding workflow.

It is the user's responsibility to validate performance for assays used in their laboratory that are not covered by the QIAGEN performance studies. To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. For further validation, the guideline of the *International Conference on Harmonization of Technical Requirements (ICH) in ICH Q2(R1) Validation Of Analytical Procedures: Text And Methodology* are recommended.

The QIAcuityDx Universal MasterMix Kit is not produced under sterile manufacturing procedures, therefore it may contain other ingredients which might influence the measurement. Downstream applications should include adequate controls if this increases the risk of a negative impact on the diagnostic result.

Troubleshooting

This section provides information about what to do in the event of problems with the use of the QIAcuityDx Universal MasterMix Kit. If further assistance is required, contact QIAGEN technical services using the contact information below, which will direct you to country specific contact details:

Website: **support.qiagen.com**

Issue	Comments and suggestions
NTC amplification	
Assay design	Redesign primers/probes. Optimize assay conditions by varying primer probe concentration and $MgCl_2$ concentration.
Contamination in reagents	Discard reagents, repeat assay using new reagents.
Contamination in assay set-up.	Take precautions against contamination by decontaminating working area using appropriate cleaning materials.
No amplification	
PCR conditions not optimized	Increase initial denaturation time. Increase annealing/extension time.
Insufficient starting template	Increase the amount/concentration of starting template added to assay master mix.
Saturation flag	
Over-saturation of probes	Decrease exposure time in the imaging parameters. Decrease the gain in the imaging parameters.
Insufficient separation between positive and negative clusters	
Assay design	Optimize assay conditions by varying primer probe concentration and $MgCl_2$ concentration. Switch to double quenched TaqMan probes to increase signal-to-noise ratio.
PCR conditions not optimized	Increase initial denaturation time. Increase annealing/extension time.
Differences observed in absolute quantification values between runs	
Insufficient addition of QIAcuityDx Universal MasterMix	Ensure the final concentration of QIAcuityDx Universal MasterMix in the sub-mix is 1x (from the 4x stock solution).

Issue	Comments and suggestions
Variation in thaw/set-up time	Extended thaw/set-up times may negatively impact absolute quantification values. For optimal performance reagents should be thawed for a maximum of 30 minutes and once the submix (assay master mix + template) is prepared, it should be immediately loaded onto the nanoplate. If extended thaw/set-up times are necessary, these should be guardbanded on a per-assay basis to ensure any changes in absolute quantification do not affect end results.
PCR conditions not optimized	Optimize denaturation temperature. Optimize annealing/extension temperature.
Inconsistent results between nanoplate wells	
PCR conditions not optimized	Optimize activation time by increasing from 2 minutes up to 15 minutes.

Ordering Information

Product	Contents	Cat. no.
QIAcuityDx Universal MasterMix Kit (1 mL)	For preparation of up to four QIAcuityDx Nanoplates: 1 x QIAcuityDx Universal MasterMix, 1 x $MgCl_2$, 200 mM, 2 x RNase-free water	260101
QIAcuityDx Universal MasterMix Kit (5 mL)	For preparation of up to twenty QIAcuityDx Nanoplates: 5 x QIAcuityDx Universal MasterMix, 2 x $MgCl_2$, 200 mM, 5 x RNase-free water	260102

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to follow any applicable local regulation and we also recommend to follow any applicable standards and guidelines.

Document Revision History

Date	Changes
R1, July 2024	Initial release

Limited License Agreement for QIAcuityDx® Universal MasterMix Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the panel only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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