

ddPCR™ Gene Expression Probe Assays

Catalog # Description

10031252 ddPCR Gene Expression Assay (FAM), 200 x 20 µL reactions
 10031253 ddPCR Gene Expression Assay (FAM), 1,000 x 20 µL reactions
 10031254 ddPCR Gene Expression Assay (FAM), 2,500 x 20 µL reactions
 10031255 ddPCR Gene Expression Assay (HEX), 200 x 20 µL reactions
 10031256 ddPCR Gene Expression Assay (HEX), 1,000 x 20 µL reactions
 10031257 ddPCR Gene Expression Assay (HEX), 2,500 x 20 µL reactions
 12005582 ddPCR Gene Expression Assay (Cy5), 200 x 20 µL reactions
 12005583 ddPCR Gene Expression Assay (Cy5), 1,000 x 20 µL reactions
 12005584 ddPCR Gene Expression Assay (Cy5), 2,500 x 20 µL reactions
 12005585 ddPCR Gene Expression Assay (Cy5.5), 200 x 20 µL reactions
 12005586 ddPCR Gene Expression Assay (Cy5.5), 1,000 x 20 µL reactions
 12005587 ddPCR Gene Expression Assay (Cy5.5), 2,500 x 20 µL reactions
 12017404 ddPCR Gene Expression Assay (ROX), 200 x 20 µL reactions
 12017425 ddPCR Gene Expression Assay (ROX), 1,000 x 20 µL reactions
 12017426 ddPCR Gene Expression Assay (ROX), 2,500 x 20 µL reactions
 12017374 ddPCR Gene Expression Assay (ATTO 590), 200 x 20 µL reactions
 12017427 ddPCR Gene Expression Assay (ATTO 590), 1,000 x 20 µL reactions
 12017394 ddPCR Gene Expression Assay (ATTO 590), 2,500 x 20 µL reactions
 12025579 ddPCR Gene Expression Assay (ATTO 550), 200 x 20 µL reactions
 12025605 ddPCR Gene Expression Assay (ATTO 550), 1,000 x 20 µL reactions
 12025596 ddPCR Gene Expression Assay (ATTO 550), 2,500 x 20 µL reactions

For Research Use Only. Not for use in diagnostic procedures.

Description

ddPCR Gene Expression Probe Assays are designed for maximum specificity and transcript coverage. These assays are available with a FAM, HEX, Cy5, Cy5.5, ROX, ATTO 590, or ATTO 550 fluorophore for the human, mouse, and rat genomes. All of the assays can be used with Droplet Digital™ PCR (ddPCR) Systems.

Ordering Information

Visit bio-rad.com/digital-assays to order ddPCR Gene Expression Probe Assays.

Storage and Stability

ddPCR Gene Expression Probe Assays are stable for 12 months when stored at 4°C protected from light. The assay mix can be kept at -20°C for long-term storage.

Kit Contents

The ddPCR Gene Expression Probe Assay is a 20x concentrated, ready-to-use primer-probe mix. Each kit comes with 200, 1,000, or 2,500 µL of the 20x assay mix (18 µM primers and 5 µM probe) sufficient for 200, 1,000, or 2,500 x 20 µL reactions, respectively. For assays performed on the QX700™ ddPCR System, this would correspond to four times the number of reactions listed below, as based on a final sample input volume of 5 µL in RDG16 Cartridges.

See Table 1 for a list of fluorophores compatible with your system.

Visit bio-rad.com/ddPCRconsumables to find ordering information on consumables such as oils, cartridges, gaskets, plates, and seals.

Table 1. Fluorophore compatibility.

QX200™ Droplet Reader	QX ONE™ ddPCR System	QX Continuum™ ddPCR System	QX600™ Droplet Reader	QX700 Droplet Digital PCR System
FAM	FAM	FAM	FAM	FAM
HEX	HEX	HEX	HEX	HEX
	Cy5	ROX	ROX	ATTO 550
	Cy5.5	Cy5	ATTO 590	ROX
			Cy5	ATTO 590
			Cy5.5	Cy5
				Cy5.5

QX200, QX600, and QX ONE Droplet Digital PCR Systems

Reagents and Equipment

For assays using the QX200 Droplet Generator (catalog #1864002) or Automated Droplet Generator (#1864101):

- iScript Advanced cDNA Synthesis Kit for RT-qPCR (#1725037, #1725038)
- For 1–2 targets, ddPCR Supermix for Probes (No dUTP) (#1863023, #1863024, #1863025) is recommended
- For >2 targets, ddPCR Multiplex Supermix (#12005909, #12005910, #12005911) is recommended
- QX200 Droplet Reader (#1864003) or QX600 Droplet Reader (#12013328)
- PX1 PCR Plate Sealer (#1814000)

For assays using the QX ONE Droplet Digital PCR System (#12006536):

- iScript Advanced cDNA Synthesis Kit for RT-qPCR (#1725037, #1725038)



- ddPCR Multiplex Supermix (#12005909, #12005910, #12005911)
- PX1 PCR Plate Sealer (#1814000)

Protocol

cDNA Synthesis

Prepare cDNA with iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the recommended protocol in the product insert (10000070791).

cDNA Amount to Include in Droplet Digital PCR Mix

Up to 6 μL cDNA (not exceeding the equivalent of 50 ng initial RNA) can be used per ddPCR reaction. Generally, the cDNA resulting from 1 ng RNA is sufficient for detection of most transcripts. However, dilutions may be required for abundant transcripts.

Droplet Digital PCR Mix Setup

1. Thaw all frozen reaction components to room temperature. Mix thoroughly by vortexing the tube to ensure homogeneity because a concentration gradient may form during -20°C storage. Centrifuge briefly to collect contents at the bottom of the tube.
2. Prepare samples at room temperature according to the recommendations in Table 2. If multiple samples are to be assayed using the same target and reference, prepare a master reaction mix without the sample template, dispense equal aliquots into the reaction tubes, and add the sample template to each reaction tube as the final step. An example reaction setup for the QX200, QX ONE, or QX600 ddPCR System is provided in Table 4. For instructions on reaction setup for the QX Continuum Droplet Digital PCR System, refer to the QX Continuum ddPCR Supermix for Probes Product Insert (10000167515).
3. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow the reaction tube to equilibrate at room temperature for about 3 min.
4. Transfer the reaction mix from the reaction tubes to the appropriate Droplet Digital PCR cartridge as follows:
 - For the QX200 Droplet Generator, load 20 μL of each reaction mix into the sample wells of DG8 Cartridges. Follow subsequent instructions as specified in the QX200 Droplet Generator Instruction Manual (10031907)
 - For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (10043138)
 - For the QX ONE Droplet Digital PCR System, load 20 μL of each reaction mix into the sample wells of GCR96 Cartridges. Follow subsequent instructions as specified in the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide (10000116512)

Table 2. Preparation of the reaction mix for single and multiplex assays. Example setup for QX ONE, QX200, and QX600 ddPCR Systems.

Component	Volume per reaction, μL	Final concentration
2x ddPCR Supermix for Probes (No dUTP)	11	1x
20x target primers/probe (Dye 1)	1.1	900 nM primers/ 250 nM probe
20x reference primers/probe (Dye 2)	1.1	900 nM primers/ 250 nM probe
cDNA with iScript Advanced cDNA Synthesis Kit for RT-qPCR	Up to 6	Up to 50 ng initial RNA
RNase-/DNase-free water	Variable	—
Total volume	22	—

Thermal Cycling Conditions

Follow instructions for thermal cycling based on the droplet generator used:

- For the QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate. Seal the plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling (see Table 3)
- For the QX600 Automated Droplet Generator, remove the droplet plate containing ddPCR droplets from the generator. Seal the plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling (see Table 3)
- For the QX ONE Droplet Digital PCR System, thermal cycling is integrated into and sequentially performed by the system itself. Hence, no additional equipment or sample handling is required for this step. Refer to the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide (10000116512) for plate setup instructions, and use appropriate thermal cycling conditions, as specified in Table 3

Table 3. Thermal cycling conditions for the QX200, QX600, and QX ONE Droplet Digital PCR Systems.

Cycling step	Temperature, $^{\circ}\text{C}^{**}$	Time	Number of cycles
Hold (QX ONE ddPCR System only)	25	3 min	1
Enzyme activation	95	10 min	1
Denaturation	94	30 sec	40
Annealing/extension	55	1 min**	40
Enzyme deactivation	98	10 min	1
Hold	25	1 min	1

* For the C1000 Touch Thermal Cycler, use a heated lid set to 105°C and set the sample volume to 40 μL .

** Check/adjust ramp rate settings to $\sim 2^{\circ}\text{C}/\text{sec}$.

The QX Continuum System

For assays using the QX Continuum System (#12019613):

- iScript Advanced cDNA Synthesis Kit for RT-qPCR (#1725037, #1725038)

- QX Continuum ddPCR Supermix for Probes (#12019028, #12019141, #12019133)
- PX1 PCR Plate Sealer (#1814000)

Protocol

cDNA Synthesis

Prepare cDNA with iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the recommended protocol in the product insert (10000070791).

cDNA Amount to Include in Droplet Digital PCR Mix

Up to 6 µL cDNA (not exceeding the equivalent of 50 ng initial RNA) can be used per reaction. Generally, the cDNA resulting from 1 ng RNA is sufficient for detection of most transcripts. However, dilutions may be required for abundant transcripts.

Droplet Digital PCR Mix Setup

1. Thaw all frozen reaction components to room temperature. Mix thoroughly by vortexing the tube to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of the tube.
2. Prepare samples at room temperature according to the recommendations in Table 4. If multiple samples are to be assayed using the same target and reference, prepare a master reaction mix without sample template, dispense equal aliquots into the reaction tubes, and add the sample template to each reaction tube as the final step. An example reaction setup for the QX Continuum System is shown in Table 4.
3. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow the reaction tube to equilibrate at room temperature for about 3 min.
4. Transfer the reaction mix from the reaction tubes to the appropriate Droplet Digital PCR cartridge, loading 16 µL of each mix into the sample wells of Hard-Shell™ 96-Well PCR Plates (#HSP9601). Refer to the QX Continuum Droplet Digital PCR System Instruction Manual (10000170603) for additional details.

Table 4. Example of an assay on the QX Continuum System.

Component	Volume per reaction, µL	Final concentration
4x ddPCR Supermix for Probes	4.5	1x
20x target 1 primers/probe (Dye 1)	0.9	900 nM/250 nM
20x target 2 primers/probe (Dye 2)	0.9	900 nM/250 nM
20x target 3 primers/probe (Dye 3)	0.9	900 nM/250 nM
20x target 4 primers/probe (Dye 4)	0.8	900 nM/250 nM
Diluted restriction enzyme (see DNA digestion section)	1.125	2–5 units*
Sample	Variable	Up to 16.5 ng/µL**
RNase-/DNase-free water	Variable	—
Total volume	18	—

* For DNA ≤3.3 ng/µL, restriction enzyme digestion may not be necessary, and no incubation is needed for digestion. The reaction mixture can be loaded into the QX Continuum System immediately.

** Maximum input should be ≤264 ng per reaction.

With the QX Continuum Droplet Digital PCR System, thermal cycling is integrated into and sequentially performed by the system itself. Hence, no additional equipment or sample handling is required for this step.

For thermal cycling conditions on the QX Continuum Droplet Digital PCR system, refer to Table 5. Thermal profiles that run on the QX Continuum System are created in QX Insight Software. Thermal profiles are configured for 40 cycles and contain a default temperature array, which can be adjusted according to needs.

Table 5. Thermal cycling conditions for the QX Continuum Droplet Digital PCR System.

Thermal cycling zone	Recommended temperature (°C)
Reverse transcription	50
Activation	95
Denaturation	95
Annealing	60
Extension	60

The QX700 Droplet Digital PCR System

For assays using QX700 Droplet Digital PCR Systems (#17011036, #17010638, #17010628):

- iScript Advanced cDNA Synthesis Kit for RT-qPCR (#1725037, #1725038)
- naica™ 5X Multiplex ddPCR Mix (#12025253, #12025254)
- naica 10X Multiplex ddPCR Mix (#12025255, #12025256, #12025258)
- PX1 PCR Plate Sealer (#1814000)

Reaction Protocol

For reaction assembly with the QX700 Droplet Digital PCR System, see Table 6.

1. Thaw Buffer A completely before each use. Vortex thoroughly (suggested three times for 5–10 sec each at maximum speed) and briefly centrifuge to collect the liquid at the bottom of the tube.
2. For Buffer B, it is recommended to start with a final concentration of 4% and not to exceed 5% during assay optimization. Typical final concentrations range from 2 to 5%.
3. Vortex the primers and probes thoroughly before use. After combining all reagents, vortex thoroughly (suggested 5–10 sec at maximum speed) to mix the contents. Centrifuge briefly to collect the liquid at the bottom of the tube before loading the reaction mix onto consumable chips; immediately load the reaction on the respective chip. It is not recommended to freeze the combined reagent solution. For RDG16 Cartridges, the final well reaction volume is 5 µL.

Thermal Cycling

See Table 7 for thermal cycling conditions using the QX700 Droplet Digital PCR System.

Table 6. Reaction assembly for the QX700 Droplet Digital ddPCR System.

Component	Final concentration	Volume per reaction, μL	
		5X Buffer A	10X Buffer A
naica Multiplex ddPCR Mix Buffer A	1x	1.4	0.7
naica Multiplex ddPCR Mix Buffer B	4%*	0.28	0.28
20x Target 1 primers/probe (Dye 1)**	1x	0.35	0.35
20x Target 2 primers/probe (Dye 2)**	1x	0.35	0.35
cDNA with iScript Advanced cDNA Synthesis Kit for RT-qPCR	Up to 50 ng initial RNA	Variable	Variable
RNase-/DNase-free water	—	Variable	Variable
Total volume		7	7

* Suggested final concentration, not to exceed 5%. Buffer B is provided at an initial concentration of 100%.

** Additional primers/probe sets with different dyes may be added according to Table

Table 7. QX700 Droplet Digital PCR System thermal cycling conditions.

Steps*	Temperature ($^{\circ}\text{C}$)	Time
Step 1	95	180 sec
Step 2	Begin loop for 45 iterations	
Step 3	95	10 sec
Step 4	55	15 sec

*Use a ramp rate of $1^{\circ}\text{C}/\text{sec}$ for each step.

Use QX700 ddPCR System Analysis Software to edit/modify the thermal cycling conditions.

Data Acquisition and Analysis

Follow instructions for data acquisition and analysis based on the droplet reader used:

- For the QX200 Droplet Reader, refer to the QX200 Droplet Reader and QX Manager Software Standard Edition User Guide (10000107223) or the QX200 Droplet Reader and QX Manager Software Premium Edition User Guide (10000163733)
- For the QX600 Droplet Reader, refer to the QX600 Droplet Reader and QX Manager Software Standard Edition User Guide (10000153877) or the QX600 Droplet Reader and QX Manager Software Premium Edition User Guide (10000153878)
- For the QX ONE Droplet Digital PCR System, refer to the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide (10000116512) and the QX ONE Software User Guide for Standard Edition (10000116655) or Regulatory Edition (10000116656)
- For the QX Continuum Droplet Digital PCR System, refer to the QX Continuum Droplet Digital PCR System Instruction Manual (10000170603)
- For the QX700 ddPCR System, refer to the QX700 System Analysis Software User Guide (10000171494)

Quality Control

ddPCR Gene Expression Probe Assays are free of detectable DNase and RNase activities. Stringent specifications are maintained to ensure lot-to-lot consistency.

Visit bio-rad.com/digital-assays for more information.

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