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

For research purposes only.

Description

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

Ordering Information

ddPCR Gene Expression Probe Assays can be ordered only online at bio-rad.com/digital-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.

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)

Refer to the QX200 Droplet Generator Instruction Manual (10031907), QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512), or Automated Droplet Generator Instruction Manual (10043138) for ordering information on consumables such as oils, cartridges, gaskets, plates, and seals. See Table 1 for a list of fluorophores compatible with your system.

Table 1. Fluorophore compatibility.

QX200 Droplet Reader	QX ONE ddPCR System	QX600 Droplet Reader
FAM	FAM	FAM
HEX	HEX	HEX
	Cy5	Cy5
	CY5.5	Cy5.5
		ROX
		ATTO 590

Protocol

cDNA Synthesis

Make cDNA with iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the recommended protocol in the product insert (10042279).

cDNA Amount to Include in ddPCR Reaction Mix

Up to 6 µl cDNA (not exceeding the equivalent of 50 ng initial RNA) can be used per ddPCR reaction (20 µl final volume). Generally, the cDNA resulting from 1 ng RNA is sufficient for detection of most transcripts. However, dilutions may be required for abundant transcripts.

ddPCR Reaction 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 tubes.
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 sample template, dispense equal aliquots into the reaction tubes, and add the sample template to each reaction tube as the final step.

Table 2. Preparation of the reaction mix for single and multiplex assays.

Component	Volume per Reaction, µl	Final Concentration
2x ddPCR Supermix for Probes (No dUTP)	10	1x
20x target primers/probe (FAM)	1	900 nM primers/ 250 nM probe
20x reference primers/probe (HEX)	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	**20***	**—**

* For the Automated Droplet Generator, prepare 22 µl per well.

3. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow reaction tubes to equilibrate at room temperature for about 3 minutes.
4. Transfer the reaction mix from the reaction tubes to the appropriate ddPCR Cartridge as follows:
   - For the QX200 Droplet Generator, load 20 µl of each reaction mix into a sample well of a DG8 Cartridge. 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 a sample well of a GCR96 Cartridge. Follow subsequent instructions as specified in the QX ONE Droplet Digital PCR System and QX ONE Software Instruction Manual (10000116512)

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 Automated Droplet Generator, remove the droplet plate containing ddPCR droplets from the Automated Droplet 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 Instruction Manual (10000116512) for plate setup instructions. Use appropriate thermal cycling conditions as specified in Table 3

Table 3. Thermal cycling conditions.*

Cycling Step		Temperature, °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	QX200 or QX600 ddPCR System (optional)	4	Infinite	1
	QX ONE ddPCR System (required)	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 ~2°C/sec.

Data Acquisition and Analysis

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

- 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 Regulatory Edition User Guide (10000107224)
- 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 Instruction Manual (10000116512) and the QX ONE Software User Guide for Standard Edition (10000116655) or Regulatory Edition (10000116656)

Other Recommendations

When running technical replicate wells, assemble a common reaction mix (enough for 1.5x as many wells as you plan to run) with all required components and sample template.

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/DropletDigitalPCRAssays** for more information.

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