ddPCR Gene Expression EvaGreen® Assays

Catalog #	Description
10031258	**ddPCR Gene Expression EvaGreen® Assay**, 200 reactions
10031259	**ddPCR Gene Expression EvaGreen® Assay**, 1,000 reactions
10031260	**ddPCR Gene Expression EvaGreen® Assay**, 2,500 reactions

For research purposes only.

Description

Droplet Digital PCR (ddPCR) Gene Expression EvaGreen® Assays enable accurate quantification of small fold changes for detecting targets that may be present at low levels or substances in samples that can inhibit a PCR reaction.

Ordering Information

Go to **bio-rad.com/digital-assays** to order ddPCR Gene Expression EvaGreen® Assays.

Storage and Stability

The real-time PCR primer assays are stable for 12 months when stored at 4°C. The 20x assay mix can be kept at –20°C for long-term storage.

Kit Contents

The real-time PCR primer assay consists of unlabeled PCR primer pairs for use with dye-based chemistry, such as the QX200 ddPCR EvaGreen® Supermix.

Other Required Materials and Instruments

- iScript Advanced cDNA Synthesis Kit for RT-qPCR (catalog #1725037 and 1725038)
- QX200 ddPCR EvaGreen® Supermix (#1864033, 1864034, 1864035, and 1864036)
- QX200 Droplet Generator (#1864002) or Automated Droplet Generator (#1864101)
- QX200 AutoDG Droplet Digital PCR System (#1864100)
- QX200 Droplet Generation Oil for EvaGreen® (#1864006)
- C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module (#1851197)
- PX1 PCR Plate Sealer (#1814000)

Refer to the instrument-specific manuals for ordering information for other consumables (oils, cartridges, gaskets, plates, and seals).

Protocol

Step 1: cDNA Synthesis with iScript Advanced cDNA Synthesis Kit for RT-qPCR

Make cDNA with iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the recommended protocol (10000070791).

Step 2: cDNA Amount to Include in ddPCR Reaction Mix

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

Step 3: ddPCR Reaction Mix Setup

1. Thaw all frozen reaction components to room temperature. Mix thoroughly, then centrifuge briefly to collect solutions at the bottom of tubes. Store the tubes on ice.
2. Prepare the sample at room temperature according to the recommendations in Table 1. If multiple samples are to be assayed using the same target, prepare a master reaction mix without sample template, dispense equal aliquots into the reaction tubes, and add the resuspension of sample DNA.

Table 1. ddPCR reaction setup.

Component	Volume per Reaction, µl	Final Concentration
2x QX200 ddPCR EvaGreen® Supermix	10	1x
20x primers	1	250 nM primers
cDNA with iScript Advanced cDNA Synthesis Kit for RT-qPCR	Up to 6	Up to 50 nm initial RNA
RNase-/DNase-free water	Variable	–
Total volume	**20**	**–**

3. Vortex the reaction mixture thoroughly, spin down, and dispense 20 µl of the mix into the sample well of the droplet generator cartridge. Follow the guidelines for droplet generation in the QX200 Droplet Generator Instruction Manual (10031907).
4. After droplet generation, transfer the reaction mix into the recommended 96-well PCR plate.
5. Program the thermal cycling protocol on the C1000 Touch Thermal Cycler according to the guidelines in Table 2.
6. Load the PCR plate onto the thermal cycler and start the PCR run. After thermal cycling, transfer the PCR reaction plate onto a QX200 Droplet Reader and follow the instrument guidelines.

Table 2. Thermal cycling protocol.*

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	5 min	~2°C/sec	1
Denaturation	96	30 sec		40
Annealing/extension	58	1 min		40
Signal stabilization	4	5 min		1
	90	5 min		1
Hold (optional)	4	Infinite	–	1

* Use a heated lid set to 105°C and set the sample volume to 40 µl.

Other Recommendations

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

- Run at least 1 negative control
- Run a positive control at a concentration similar to the unknown samples

Quality Control

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

Visit **bio-rad.com/DropletDigitalPCRAssays** to learn more about Bio-Rad's Droplet Digital PCR solution.

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