

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.

Reagents and Equipment

- iScript Advanced cDNA Synthesis Kit for RT-qPCR (catalog #1725037, #1725038)
- QX200 ddPCR EvaGreen® Supermix (#1864033, #1864034, #1864035, #1864036)
- QX200 Droplet Generator (#1864002) or Automated Droplet Generator (#1864101)
- QX200 Droplet Reader (#1864003) or QX600™ Droplet Reader (#12013328)
- 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 in the product insert (10042279).

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* | – |

* 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)

5. 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 2)
 - 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 2)

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.

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)

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](https://www.bio-rad.com/DropletDigitalPCRAssays) to learn more about Bio-Rad's Droplet Digital PCR solutions.



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