BIO-RAD

PrimePCR™ ddPCR™ Gene Expression Probe Assays

For research purposes only.

Description

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

Ordering Information

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

Storage and Stability

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

Kit Contents

The PrimePCR ddPCR Gene Expression Probe Assay is a 20x concentrated, ready-to-use, primer-probe mix optimized for use with ddPCR Supermix for Probes (No dUTP). 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.

Required Equipment

- QX100™ or QX200™ Droplet Generator (catalog #186-3002 or 186-4002, respectively) or Automated Droplet Generator (catalog #186-4101)
- QX100 or QX200 Droplet Reader (catalog #186-3003 or 186-4003, respectively)
- C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module (catalog #185-1197)
- PX1™ PCR Plate Sealer (catalog #181-4000)

Please refer to the QX100 or QX200 Instruction Manuals (#10026321 and 10026322 or 10031906 and 10031907, respectively) or the Automated Droplet Generator Instruction Manual (#10043138) for ordering information on consumables (oils, cartridges, gaskets, plates, and seals).

Protocol

cDNA Synthesis

Make cDNA with iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (catalog #172-5037 or 172-5038) 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 1. 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 1. Preparation of the reaction mix.

Component	Volume per Reaction, µl	Final Concentration
2x ddPCR Supermix for Probes (No dUTP)	10	1x
20x target primers/probe mix (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**	**—**

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. Once the reaction mixtures are ready, load 20 µl of each reaction mix into a sample well of a DG8™ Cartridge for QX200/QX100 Droplet Generator (catalog #186-4008) followed by 70 µl of Droplet Generation Oil for Probes (catalog #186-3005) into the oil wells, according to the QX100 or QX200 Droplet Generator Instruction Manual (#10026322 or 10031907, respectively). For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (#10043138).

Thermal Cycling Conditions and Data Acquisition

1. After droplet generation with the QX100 or QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate. For the Automated Droplet Generator, remove the droplet plate containing ddPCR droplets. Seal the plate with the recommended foil seal and a PX1 PCR Plate Sealer.
2. Perform thermal cycling of droplets using a C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module according to the protocol shown in Table 2.
3. After thermal cycling, place the sealed plate in a QX100 or QX200 Droplet Reader. Follow the guidelines in the QX100 or QX200 Droplet Reader and QuantaSoft Software Instruction Manual (#10026321 or 10031906, respectively).

Table 2. Thermal cycling protocol.*

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	2°C/sec	1
Denaturation	94	30 sec		40
Annealing/extension	55	1 min		
Enzyme deactivation	98	10 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 1.5x as many wells as you plan to run) with all required components and sample template.

Quality Control

PrimePCR 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/web/ddPCRprobeassays** for more information.

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