PrimePCR™ ddPCR™ Gene Expression Assays (EvaGreen®)

For research use only. Not for diagnostic purposes.

Ordering Information
PrimePCR assays for ddPCR can be ordered only online at www.bio-rad.com/primepcr.

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. Individual assays are provided as a 20x stock solution of 200, 1,000, or 2,500 reactions.

Other Required Material and Instruments
iScript™ advanced cDNA synthesis kit (catalog #170-8842, 170-8843)
QX200 ddPCR EvaGreen supermix (catalog #186-4033, 186-4034, 186-4035,186-4036)
20x real-time PCR primer assay
QX200 droplet generator (catalog #186-4002)
QX200 droplet reader (catalog #186-4003)
QX200 droplet generation oil for EvaGreen (catalog #186-4006)
QX100™/QX200 droplet reader oil (catalog #186-3004)
C1000 Touch™ thermal cycler (catalog #185-1196)
PX1™ PCR plate sealer (catalog #181-4000)
Please refer to 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
- Make cDNA with iScript advanced cDNA synthesis kit according to the recommended protocol (PN 10022089 Rev A)

Step 2: cDNA amount to include in ddPCR reaction mix
- Up to 6 µl of 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 of RNA is sufficient for detection of most transcripts

Step 3: ddPCR reaction mix setup
- Thaw all frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, and then store on ice
- Prepare samples at room temperature according to 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 sample template to each reaction tube as the final step. Mix by pipetting up and down 5–10 times to ensure complete resuspension of sample DNA

Table 1. ddPCR Reaction Setup

Component	Volume per Reaction	Final Concentration
2x QX200 ddPCR EvaGreen supermix	10 µl	1x
20x primers	1 µl	250 nM primers
cDNA with iScript advanced cDNA synthesis kit	up to 6 µl	up to 50 ng initial RNA
RNase/DNase-free water	Variable	—
Total volume	**20 µl**	**—**

- Vortex reaction mixture thoroughly, spin down, and dispense 20 µl of the mix into the sample well of the droplet generator cartridge. Follow general guidelines for droplet generation (#10031907)
- After droplet generation, transfer reaction mix onto the recommended 96-well PCR plate
- Program thermal cycling protocol on the C1000 Touch thermal cycler according to Table 2
- Load the PCR plate onto the thermal cycler and start the PCR run. After thermal cycling, transfer PCR reaction plate onto a QX200 droplet reader and follow instrument-specific guidelines (#10031906)

Table 2. Thermal Cycling Protocol*

Cycling Step	Temperature	Time	Ramp Rate	# Cycles
Enzyme activation	95°C	5 min	~2°C/sec	1
Denaturation	96°C	30 sec		40
Annealing/extension	58°C	1 min		
Signal stabilization	4°C	5 min		1
	90°C	5 min		1
Hold (optional)	4°C	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 2x as many wells as you plan to run) with all required components and sample template.

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

Quality Control

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

To learn more about Bio-Rad's complete solution for digital PCR, visit our website: **www.bio-rad.com/digitalPCR**.

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