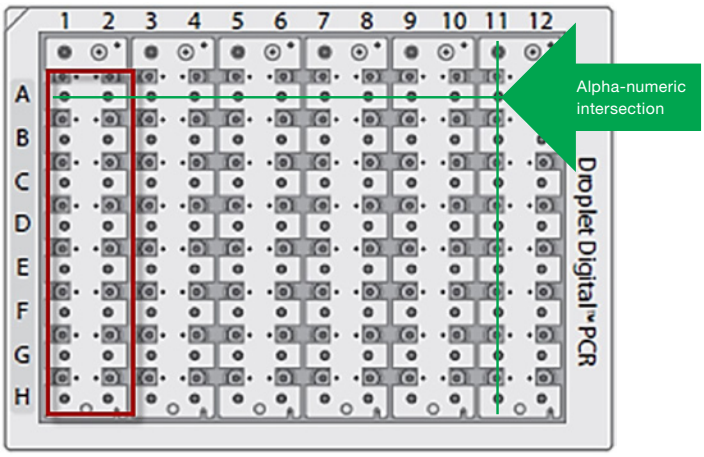
Preparing a GCR96 Cartridge for a QX ONE™ Droplet Digital™ PCR System Run

Quick Start Guide

Transferring Droplet Digital PCR (ddPCR™) Reactions to a GCR96 Cartridge

Using a Rainin LTS P20 Multichannel Pipettor, transfer 20 µl of ddPCR reactions, consisting of RNA, DNA, or cDNA template, sample, supermix, assay(s), and nuclease-free water, into the wells of a GCR96 Cartridge that are designated by the labeled alpha-numeric intersections (the wells that align with both a letter and number, as shown). Insert the pipet tips into the wells at approximately a 15° angle, contacting the bottom of the wells. Dispense the ddPCR reactions slowly (approximately 5 sec). Do not push the pipet plunger past the first stop.

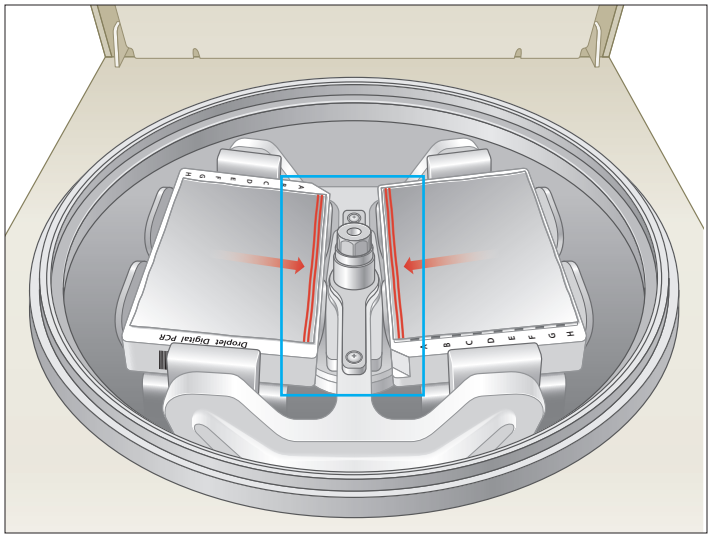
Note: Before transferring ddPCR reactions to a GCR96 Cartridge, **thoroughly mix reagents** after thawing them at room temperature (vortex supermix for 30 sec after thawing). Upon generating master mix(es) and after adding the sample/template to the master mix, vortex once more (approximately 15 sec). Centrifuge the ddPCR reactions after vortexing to collect the volume at the bottom of each well (30 sec, 1,150 rcf). Centrifugation eliminates air bubbles and ensures that the ddPCR reactions are at the bottom of each well. **Mixing or vortexing is critical to obtain homogeneity of reagents within droplets.**

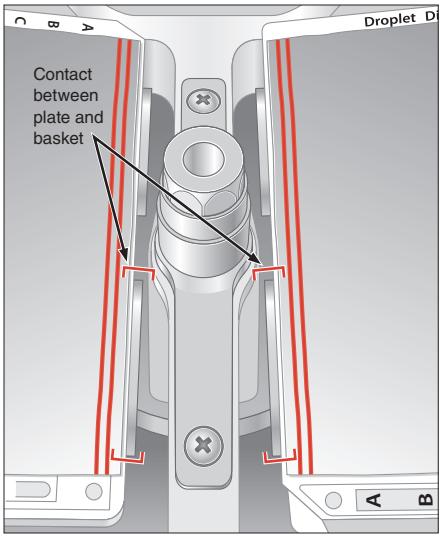


Note: GCR96 Cartridges contain six strips of 16 wells, as shown. Each strip of 16 is called a DG16 Cartridge. All DG16 Cartridges must be filled with ddPCR reactions or a 1:1 ratio of ddPCR Buffer Control for Probes (Bio-Rad™ Laboratories, Inc., catalog #1863052) and water. Failure to do so will affect the performance of the QX ONE ddPCR System and subsequent experimental data.

Sealing a GCR96 Cartridge

1. Check the PX1 PCR Plate Sealer to ensure that the correct settings are programmed for sealing the GCR96 Cartridge (Temp.: 180˚C; Time: 0.5 sec).
2. Securely load the GCR96 Cartridge on the silver heat block (Z-shaped support block; bottom protruding edge of heat block should face the operator). Orient the GCR96 Cartridge so that well A1 is at the upper left corner of the plate.
3. Place the pierceable foil heat seal with the double red stripes face up (facing toward the operator) on the GCR96 Cartridge. Ensure the foil seal covers the entire surface area of the cartridge, with the exception of the cartridge labels identifying rows A–H (left-hand side of cartridge) and the Droplet Digital PCR label (right-hand side of cartridge), and that the seal remains flush with the top and bottom of the cartridge (ensure no foil overhangs on any side).





4. Select **Eject** from the PX1 PCR Plate Sealer touch screen to eject the drawer. Load the heat block, cartridge, and seal into the PX1 Sealer. Close the drawer by selecting **Close** in the touch-screen menu on the PX1 Sealer (check to make sure seal is still aligned correctly before closing the drawer).
5. Select **Seal** (Seal will be green when the PX1 Sealer has reached the sealing temperature of 180˚C). The drawer will automatically eject when the seal is completed.
6. When the PX1 Sealer ejects the drawer, carefully rotate the semisealed cartridge 180° (do not rotate the heat block from its original orientation) and repeat steps 5–6. Then remove the sealed cartridge and heat block from the PX1 Sealer. Store the heat block on top of the PX1 Sealer until further use and execute steps 7–9 with the sealed cartridge.
7. Place the GCR96 Cartridge in a swinging bucket rotor centrifuge, placing row A closest to the rotor spindle (refer to images shown).
8. Load a counterbalance cartridge on the opposite rotor bucket.
9. Push the individual DG16 Cartridges in the GCR96 frame toward the numbered edge as far as it will go to prevent breaking the tabs of the cartridge. Centrifuge cartridges for 30 sec at 1,150 rcf. Proceed with loading your cartridge into the QX ONE ddPCR System. Refer to Initiating and Managing a QX ONE Droplet Digital PCR System Run Quick Start Guide (bulletin 3431).

Refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) for additional details.

Visit **bio-rad.com/QXONE** for more information.

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