Droplet Generation Oil for Probes

Catalog #	Description
186-3005	**Droplet Generation Oil for Probes**, 70 ml (10 x 7 ml vials), enough for 960 x 20 µl reactions

For research purposes only.

Description

Droplet generation oil for probes is used to create water (sample) in oil droplets required for Droplet Digital™ PCR (ddPCR™) analysis and is formulated for use with ddPCR supermix for probes (catalog #186-3010, 186-3026, 186-3027, and 186-3028), ddPCR supermix for probes (no dUTP) (catalog #186-3023, 186-3024, and 186-3025), and one-step RT-ddPCR kit for probes (catalog #186-3021 and 186-3022).

Following reaction preparation using ddPCR supermix, 20 µl each of up to eight prepared samples (or blanks) and droplet generation oil for probes are transferred to a DG8™ droplet generator cartridge. The loaded cartridge is covered with a DG8 gasket and placed in a QX100™ or QX200™ droplet generator. There, the samples and oil are combined within the microchannels of the cartridge to create an emulsion of ~20,000 monodispersed, nanoliter-sized droplets for each of the samples.

Additional requirements for droplet generation are listed in Table 1. For complete system requirements, refer to the QX100™ or QX200™ droplet reader and QuantaSoft™ software instruction manual (#10026321 or 10031906, respectively).

Storage and Stability

Droplet generation oil for probes is stable for 12 months when stored at a constant temperature of 25°C (room temperature).

Sample Preparation

Prepare the PCR reaction as described in the product insert for ddPCR supermix for probes (#10026235), ddPCR supermix for probes (no dUTP) (#10026868), or one-step RT-ddPCR kit for probes (#10028782).

Droplet Generation

The QX100 or QX200 droplet generator prepares droplets for up to eight samples at a time. Droplet generation takes ~2 min for each set of eight samples (~45 min for a full 96-well plate). The detailed protocol for droplet generation can be found in the QX100 or QX200 droplet generator instruction manual (#10026322 or #10031907, respectively).

Note: Droplet generation oil for probes should be used only with ddPCR supermix for probes, ddPCR supermix for probes (no dUTP), or one-step RT-ddPCR kit for probes. The use of other supermixes can lead to poor results and potential damage to the QX100 or QX200 droplet reader.

Table 1. QX100 or QX200 droplet generation components.

Component	Description	Catalog #
QX100 or QX200 droplet generator	Instrument used for droplet generation	186-3002 or 186-4002, respectively
Droplet generator cartridges and gaskets	Microfluidic DG8 cartridge used to mix sample and oil to generate droplets; DG8 gaskets seal the cartridge to prevent evaporation and apply the pressure required for droplet formation	186-4007
DG8 cartridge holder	Positions and holds the DG8 cartridge in the instrument for droplet generation	186-3051
PCR supermix	ddPCR supermix for probes ddPCR supermix for probes (no dUTP) One-step RT-ddPCR kit for probes	186-3010, 186-3026, 186-3027, 186-3028 186-3023, 186-3024, 186-3025 186-3021, 186-3022
Control	ddPCR buffer control kit	186-3052
Pipets	20 µl pipet for sample loading 50 µl pipet for droplet transfer 8-channel, 200 µl pipet for oil	Rainin L-20 Rainin L50, L8-50 Rainin L8-200
Pipet tips	Filtered	Rainin GP-L10F, GP-L200F
96-well PCR plates	twin.tec 96-well semi-skirted plates	Eppendorf 951020362
Reagent trough	Any	
Foil seal	Pierceable foil heat seal	181-4040
Plate sealer	PX1™ PCR plate sealer	181-4000
8-cap strips	Any	

1. Insert the DG8 cartridge into the holder, aligning the notch in the cartridge with the upper left corner of the holder.
2. Transfer 20 µl of each prepared sample to the sample wells (middle row) of the DG8 cartridge.
3. Dispense the droplet generation oil for probes into a reagent trough (see Table 2 for volumes required).
4. Using a multichannel pipet, fill each oil well (bottom row) with 70 µl of droplet generation oil for probes from the reagent trough.
5. Hook the gasket over the cartridge holder using the holes on both sides. The gasket must be securely hooked on both ends of the holder to ensure pressure is sufficient to generate droplets.
6. Open the QX100 or QX200 droplet generator by pressing the button on the green top and place the cartridge holder into the instrument.
7. Press the button on the top again to close the door and initiate droplet generation.
8. When droplet generation is complete, all three indicator lights are solid green and the door can be opened by pressing the button. The holder, with the DG8 cartridge still in place, can be removed.
9. Pipet 40 µl of the contents of the top wells (the droplets) into a single column of a 96-well PCR plate. Repeat for each cartridge, filling up each column across the 96-well plate.
10. To avoid evaporation, seal the PCR plate with foil immediately after transferring all the columns of droplets.
11. Begin thermal cycling (PCR) within 30 min of sealing the plate, or store the plate at 4°C for up to 4 hr prior to thermal cycling. Refer to the ddPCR supermix product insert for cycling conditions.

Table 2. Droplet generation oil requirements.

Number of Wells	Volume of Oil, µl
8	700
24	1,820
48	3,500
96	6,860

When PCR amplification is complete, remove the 96-well plate from the thermal cycler and read the droplets using the QX100 or QX200 droplet reader (follow the instructions in the QX100 or QX200 droplet reader manual).

Recovery of Amplified Material from Droplets

Generate droplets by following the standard ddPCR workflow and protocols. If the goal is to read or quantify droplets and recover material from droplets in parallel, prepare two sets of reactions, one for each application. For example, a set of eight wells in a single DG8 cartridge can be generated: four of these will be read after thermal cycling and four will not be read.

Refer to the QX100 or QX200 droplet reader manual for more details about plate setup. Once the run has finished, remove the plate and pierce the foil of the four remaining unread wells, then proceed with droplet extraction from those wells.

Alternatively, if a readout on the droplet reader is not required, DNA extraction from droplets can proceed directly following thermal cycling according to the following protocol for recovery of DNA from droplets.

1. Pipet out the entire volume of droplets and oil from a well into a 1.5 ml tube (combine replicates if desired).
2. Pipet and discard the bottom oil phase after droplets float to the top.
3. Add 20 µl of TE buffer to the tube for each well used.
4. In a fume hood, add 70 µl of chloroform to the tube for each well and cap the tube. Calculate the additional chloroform needed by multiplying by the number of combined replicate wells, if applicable.
5. Vortex at maximum speed for 1 min. Centrifuge at 15,500 g for 10 min.
6. Carefully remove the upper aqueous phase by pipetting, avoiding the chloroform phase, and transfer it to a new 1.5 ml tube.
7. Dispose of the chloroform phase appropriately.

The aqueous phase recovered from droplets contains recovered DNA as well as dNTPs, primers, and probe. If desired, visualize with an Experion™ DNA 1K chip and/or make a tenfold dilution series and requantify by ddPCR.

For more information, visit **www.bio-rad.com/DGOilProbes**.

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