ddPCR™ KRAS Screening Multiplex Kit

Catalog # 186-3506 **Supermix Volume** 2 ml (2 x 1 ml vials) **Kit Size** 200 x 20 µl reactions

For research use only. Not for diagnostic purposes.

Storage and Stability
The ddPCR Assay is stable for 18 months when stored at 4°C protected from light. The 20x assay mix and ddPCR Supermix for Probes (no dUTP) need to be kept at –20°C for long- term storage. Repeated freezing and thawing of the kit is not recommended.

Kit Contents
The PrimePCR ddPCR KRAS Screening Multiplex Kit 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 µl of the 20x assay mix (9 µM primers and 5 µM of each probe), and 2 tubes of 2x ddPCR Supermix for Probes (no dUTP), sufficient for 200 reactions. The following assays are contained within this kit: G12A (dHsaCP2500586), G12C (dHsaCP2500584), G12D (dHsaCP2500596), G12R (dHsaCP2500590), G12S (dHsaCP2500588), G12V (dHsaCP2500592) and G13D (dHsaCP2500598).

Other Required Material and Instruments
QX100™/QX200™ Droplet Generator (catalog #186-3002/186-4002)
QX100/QX200 Droplet Reader (catalog #186-3003/186-4003)
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 or #10026322 and #10031906 or #10031907, respectively) for ordering information for other consumables (oils, cartridges, gaskets, plates, and seals).

Protocol
Step 1: Restriction Digest of Sample DNA
- DNA fragmentation by restriction digestion prior to droplet generation enables optimal accuracy by separating tandem gene copies, reducing sample viscosity, and improving template accessibility. We recommend 2-5 units per well of MseI.
- When using FFPE tissue, uracil DNA glycosylase treatment is recommended to prevent false positives
- Two strategies may be used to perform restriction digestion of DNA samples: digestion directly in the ddPCR reaction during setup, or conventional digestion prior to ddPCR

Digestion in ddPCR reaction
- Efficient digestion of sample DNA can be achieved by direct addition of restriction enzymes to the ddPCR reaction
- Approximately 2–5 units of restriction enzyme per 20 µl ddPCR reaction are recommended
- Dilute the restriction enzyme using the recommended diluent buffer according to manufacturer instructions, and add 1µl to the ddPCR reaction as outlined in Table 1. Reaction can be set up at room temperature and no additional incubation time is required
- The addition of restriction enzyme buffers with high salt can inhibit ddPCR and should be avoided

Digestion prior to ddPCR
- Restriction enzyme digestion can be carried out as a separate reaction before ddPCR reaction setup
- Use 10–20 enzyme units per microgram of genomic DNA
- Incubate the reaction for 1 hour at the temperature recommended for the restriction enzyme
- Heat inactivation is not required, but can be considered if long-term storage is required; do not heat inactivate above 65°C
- DNA purification is not necessary after restriction digestion
- Use a minimum 10-fold dilution of the digest to reduce the salt content of the sample in ddPCR
- Store digested DNA at –20°C or below

Table 1. KRAS mutation screening ddPCR reaction setup.

Component	Volume per Reaction, µl	Final Concentration
2x ddPCR Supermix for Probes (no dUTP)	10	1x
20x multiplex primers/probes (FAM + HEX)	1	900 nM primers/250 nM probes
Restriction enzyme MseI , diluted*	1	2–5 U per reaction
Uracil DNA glycosylase (UDG),**	1	2–5 U per reaction
DNA sample/water	Variable	50 fg to 100 ng***
Final volume	**20**	**--**

* When not digesting directly in the ddPCR reaction setup, this component can be replaced by water.
** When using FFPE DNA, we recommend UDG treatment to prevent false positives. This component can be replaced with water in all other cases.
*** For most routine mutation detection applications, up to 130 ng of human genomic DNA can be added per ddPCR well for a final concentration of 2,000 copies/µl.

Step 2: KRAS Mutation Screening ddPCR Reaction Setup

- Thaw all frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, and store protected from light
- Prepare samples at room temperature according to recommendations in Table 1. If multiple samples are to be assayed using the same target and wild-type duplex, 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 full addition of sample DNA
- Vortex the reaction mixture thoroughly, spin down, and dispense 20 µl of the mix into the sample well of the QX100/QX200 Droplet Generator cartridge. Follow general guidelines for droplet generation
- After droplet generation using a QX100/QX200 droplet generator, transfer the reaction mix into the recommended 96-well PCR plate
- Heat seal the plate with foil seal using the PX1 PCR Plate Sealer for 5 sec at 180°C
- Program the thermal cycling protocol on the C1000 Touch Thermal Cycler with 96–deep well reaction module (#185-1197), according to Table 2
- Load the PCR plate into the thermal cycler and start the PCR run. After thermal cycling, transfer the PCR reaction plate into a QX100/QX200 Droplet Reader and follow instrument-specific guidelines

Table 2. Thermal cycling protocol.*

Cycling Step	Temperature, °C	Time	Ramp Rate	# 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°C/sec	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.

- Run **at least** one mutation-negative control containing only wild-type DNA at a concentration similar to the unknown samples
- Run a mutation-positive control with a wild-type DNA background at a concentration similar to the unknown samples (for example, a mix of 7 ng of mutant DNA in a background of 130 ng wild-type DNA)
- For more specific recommendations and protocols, see the Mutation Detection Best Practices Guidelines and the Applications Guide (Bulletin 6407; www.bio-rad.com/web/ddPCR AppGuide).

Quality Control

PrimePCR ddPCR Mutation Detection 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 digital PCR assays, visit **www.bio-rad.com/ddPCRKRASSCMX**.

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