BIO-RAD

QX700™ ddPCR™ *KRAS* Screening Kit

Catalog #	Description
17011484	QX700 ddPCR *KRAS* Screening Kit, 800 x 5 µL reactions

The QX700 ddPCR *KRAS* Screening Kit and QX700 ddPCR System are for Research Use Only. Not for use in diagnostic procedures.

The QX700 ddPCR *KRAS* Screening Kit is designed to screen for the seven most common mutations in codons 12 and 13 of the *KRAS* gene (G12A, G12C, G12D, G12R, G12S, G12V, and G13D) and the wild-type (WT) gene in a single reaction using Droplet Digital™ PCR (ddPCR) in research settings.

Storage and Stability

QX700 ddPCR *KRAS* Screening Assay and QX700 ddPCR Supermix for Probes are stable through the expiration date printed on the product labels when stored at −20°C and protected from light. QX700 ddPCR Supermix for Probes remains liquid at −20°C and does not freeze solid.

Kit Contents

The duplex screening assay is a 20x concentrated, ready-to-use primer-probe mix; QX700 ddPCR Supermix for Probes is a 5x concentrated, ready-to-use supermix. Each kit comes with one tube of 20x assay mix and 8 tubes of 5x QX700 ddPCR Supermix for Probes, sufficient for 800 reactions. The following assays can be ordered individually for subsequent studies:

- G12A (dHsaCP2500586)
- G12C (dHsaCP2500584)
- G12D (dHsaCP2500596)
- G12R (dHsaCP2500590)
- G12S (dHsaCP2500588)
- G12V (dHsaCP2500592)
- G13D (dHsaCP2500598)

Reagents and Equipment

See Table 1 for required materials that are not included in the kit.

Table 1. Additional required materials.

Droplet Digital PCR (ddPCR) System	Consumables and Reagents

• One of the following:
  • QX700 E Droplet Digital™ PCR System (Bio-Rad Laboratories, Inc., catalog #17011036)
  • QX700 S Droplet Digital™ PCR System (#17010638)
  • QX700 HT Droplet Digital™ PCR System (#17010628)
• QX700 ddPCR System Control Software, Version 1.5 or above
• QX700 ddPCR System Analysis Software, Version 1.5 or above
	• MseI restriction enzyme
	• RDG16 Cartridges, pack of 12 (#12025252)
	• Staticide SW12 Anti-Static Wipes (#SW12)
	• Kimtech Science Precision Wipes (Kimberly Clark, catalog #34155)

Protocol

Step 1: Restriction Enzyme Digestion of Research 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. Two strategies may be used to perform restriction digestion of research samples: digestion directly in the ddPCR mix during setup (recommended) or conventional digestion prior to ddPCR setup.

Digestion Directly in the ddPCR Mixture

- Efficient digestion of research sample DNA can be achieved by direct addition of restriction enzymes to the ddPCR mix
- 3 units of MseI restriction enzyme per 5 µL Droplet Digital PCR is recommended
- Reactions 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 Droplet Digital PCR and should be avoided

Digestion Prior to Droplet Digital PCR

- Restriction enzyme digestion can be carried out as a separate reaction before ddPCR setup
- Use 10–20 units of MseI per microgram of genomic DNA
- Incubate the reaction for 1 hr 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 the ddPCR mixture
- Store digested DNA at –20°C or below
- Prepare the ddPCR mix as outlined in Table 2, without the restriction enzyme

Step 2: Reaction and Cartridge Setup

1. Thaw all components to room temperature. Mix thoroughly by vortexing each tube to ensure homogeneity, as a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of the tubes.
2. Prepare research samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix for the number of reactions needed according to the guidelines in Table 2. Assemble all required components except the research sample and dispense equal aliquots into each reaction tube. As the final step, add the research sample to each reaction tube.

Table 2. Reaction mix preparation.

Components	Volume per Reaction, µL	Final Concentration
5x QX700 ddPCR Supermix for Probes	1	1x
20x ddPCR *KRAS* G12/G13 Screening Assay	0.25	1x
MseI restriction enzyme*	Variable	3 U per reaction
Research DNA sample/water	Variable	Up to 132 ng
Total Volume**	**5**	–

*When not digesting directly in the ddPCR setup, this component can be replaced by water.

**Prepare a total reaction volume greater than 5 µL to ensure complete transfer to RDG16 Cartridges; 20% overage is recommended to account for pipetting loss.

4. Mix the assembled reaction thoroughly by vortexing the tubes. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes.
5. Load 5 µL of each reaction mix into a sample chamber of RDG16 Cartridges. Refer to QX700 Droplet Digital PCR System Instrument Guide (10000258608) and instructions for RDG16 Cartridges (10000171484) for sample and plate setup instructions.

Step 3: Experiment Setup and QX700 Droplet Digital PCR System Run

QX700 ddPCR *KRAS* G12/G13 Screening Kit is compatible with QX700 ddPCR System Control Software v1.5 and above.

Experiment setup for each QX700 ddPCR System run requires the use of three files: a protocol file, an assay file, and an experiment file. A premade protocol file and assay file for the kit are available for download from the product page. These two files contain the thermocycling and acquisition settings specific to QX700 ddPCR *KRAS* G12/G13 Screening Kit. Users must create a new experiment file for each run.

For in-depth instructions on protocol file, assay file, and experiment file set up on QX700 ddPCR Systems, refer to QX700 Droplet Digital PCR System Instrument Guide (10000258608).

Protocol File Setup

1. Select **QX700 ddPCR Supermix for Probes** from the Mix Type dropdown menu and click **Apply**.
2. Define the thermocycling program according to Table 3.

Table 3. Thermocycling conditions for QX700 ddPCR *KRAS* Screening Kit.

Cycling Step	Temperature, °C	Time, sec	Cycles	Ramp Rate, °C/sec
Enzyme activation	95	600	1	2
Denaturation	95	15	40	
Annealing/extension	53	60		

3. Define the reading program according to Table 4. Ensure the FAM (blue), HEX (teal), and Cy5 (red) channel toggle switches are positioned to the right (enabled). Position the toggle switches for all other channels to the left (disabled). The Cy5 (red) channel will not be assigned to a target but must be enabled for acquisition.

Table 4. Enabled channels with associated fluorophores and exposure time.

Dye Name	Channel	Exposure Time, ms
FAM	Blue	85
HEX	Teal	273
Cy5	Red	51

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Assay File Setup

1. Under the Assay Structure section, ensure that the three channels listed in Table 5 are toggled to the right (enabled). Position the toggle switches for all other channels to the left (disabled). The kit targets are detected in the FAM (blue) and HEX (teal) channels, as shown in Table 5.

   Note: The dyes used in the assay file should match the dyes used in the protocol file.

Table 5. Assay structure details.

Dye Name	Channel	Associated Target
FAM	Blue	Mutant
HEX	Teal	Wild type
Cy5	Red	Not used

2. The spillover compensation can be manually adjusted in the Result file after the data have been collected. Perform spillover compensation according to QX700 ddPCR System Analysis Software, Standard Edition User Guide (10000258610) to optimize cluster orientation and achieve orthogonal separation between cluster populations. Alternatively, a previously created compensation matrix may be loaded in the Spill-over compensation matrix section by selecting **Load compensation matrix from file (.ncm)**.

Experiment File Setup and Run Start

Refer to QX700 Droplet Digital PCR System Instrument Guide (10000258608).

Quality Control, Data, and Post-Processing Analysis

1. Open the data file and navigate to the QUALITY CONTROL tab. Review the quality flags, chamber images, and Nb Analyzable Droplets (droplet counts) and exclude problem chambers from analysis. Refer to QX700 ddPCR System Analysis Software, Standard Edition User Guide (10000258610) for explanations of quality flags and detailed instructions on performing quality control.
2. Navigate to the ANALYZE DATA tab, Plots & Populations heading, then the 2D dot plot subheading.
3. Under the Thresholding Mode section, select **Polygons** under Type and **Common for all chambers** under Definition Scope. The Population Axis of the Vertical Axis and Horizontal Axis may be modified by clicking the blue gear icon at the bottom left of each 2D dot plot.
4. Select all valid wells. Click and hold Ctrl on the keyboard and simultaneously right click with the mouse cursor to draw a polygon around a positive cluster. Right click multiple times to draw the desired threshold shape. To complete the cluster, stop holding Ctrl and right click.
5. Assign a name to the population, such as Mutant or Wild Type, select a desired color for the population, and click **OK.**
6. Repeat steps 4–5 to draw another polygon threshold around the remaining positive cluster. See Figure 1 for an example FAM/HEX 2D dot plot. The Cy5 (red) channel is not used but will show background fluorescence.

   Note: The mutant target is detected in the FAM (blue) channel, and the wild-type target is detected in the HEX (teal) channel. To assign populations appropriately, thresholding regions of the populations may overlap across FAM and HEX double positive droplets.
7. Navigate to the Setup heading under the POST PROCESSING tab.
8. Select **Mutant Allelic Fraction (MAF)** as the Post Processing Type.
9. Select the labeled mutant target population and click **Add population to processing** under Settings.
10. Modify the reference to the wild-type population as needed and click **APPLY**.
11. Navigate to the Results heading under the POST PROCESSING tab to view the calculated MAF (%) of each research sample.

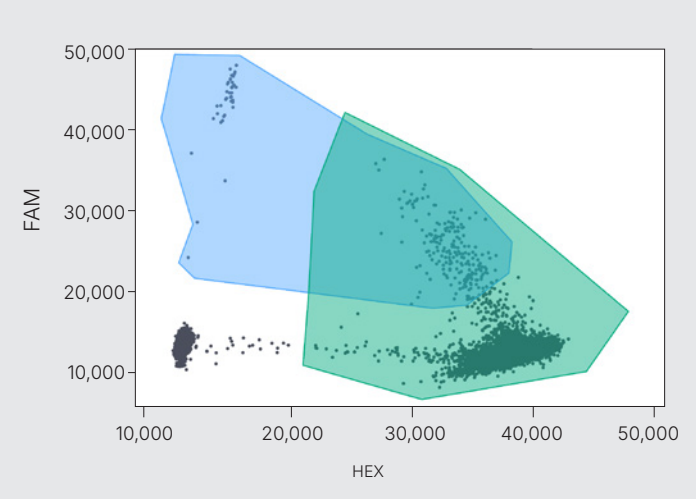

Fig. 1. Example 2D plot. Mutation-positive control with a wild-type DNA background of 132 ng.

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Recommendations for Optimal Results

- Run at least one mutation-negative control containing only wild-type DNA at a concentration similar to the unknown research samples
- Run a mutation-positive control with a wild-type DNA background at a concentration similar to the unknown research samples
- Run several no-template control (NTC) replicates
- When using formalin-fixed, paraffin-embedded (FFPE) tissue, uracil DNA glycosylase (UDG) treatment is recommended to reduce the false-positive rate and improve detection limits

  Note: FFPE DNA performance specifications have not been established, and this research sample type should be evaluated by the user as appropriate for their workflow.

Quality Control

ddPCR Mutation Detection Assays are free of detectable DNase and RNase activities. Stringent specifications are maintained to ensure lot-to-lot consistency.

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Visit **bio-rad.com/KRAS-700** for more information.

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