BIO-RAD

QX700™ ddPCR™ *EGFR* Exon 19 Deletions Screening Kit

Catalog #	Description
17011329	QX700 ddPCR *EGFR* Exon 19 Deletions Screening Kit, 800 x 5 µL reactions

The QX700 ddPCR *EGFR* Exon 19 Deletions Screening Kit and QX700 ddPCR System are for Research Use Only. Not for use in diagnostic procedures.

The QX700 ddPCR *EGFR* Exon 19 Deletions Screening Kit is designed to screen deletion mutations in exon 19 of the *EGFR* gene and the wild-type (WT) gene using Droplet Digital™ PCR (ddPCR™). Other *EGFR* exon 19 deletions present in this region may also be detected. Table 1 displays the 21 deletions that have been wet-lab validated.

Table 1. *EGFR* exon 19 deletions wet-lab validated with QX700 ddPCR *EGFR* Exon 19 Deletions Screening Kit.

COSMIC ID	Amino Acid Mutation	Coding Mutation
COSM13551	p.E746_T751delinsI	c.2235_2252>AAT
COSM6223	p.E746_A750del	c.2235_2249del15
COSM6225	p.E746_A750del	c.2236_2250del15
COSM12419	p.L747_T751delinsQ	c.2238_2252>GCA
COSM6220	p.E746_S752delinsD	c.2238_2255del18
COSM51527	p.L747_T751>Q	c.2239_2253>CAA
COSM12383	p.L747_T751delinsP	c.2239_2251>C
COSM12387	p.L747_P753delinsQ	c.2239_2258>CA
COSM12420	p.L747_T751>Q	c.2239_2252>CA
COSM6255	p.L747_S752del	c.2239_2256del18
COSM12382	p.L747_A750delinsP	c.2239_2248TTAAGAGAAG>C
COSM6218	p.E746_R748del	c.2239_2247del9
COSM12369	p.L747_T751del	c.2240_2254del15
COSM12370	p.L747_P753delinsS	c.2240_2257del18
COSM28517	p.E746_E749del	c.2235_2246del
COSM12367	p.E746_S752delinsA	c.2237_2254del18
COSM12384	p.E746_S752delinsV	c.2237_2255>T
COSM12728	p.E746_T751del	c.2236_2253del18
COSM12678	p.E746_T751delinsA	c.2237_2251del15
COSM12422	p.L747_A750delinsP	c.2238_2248>GC
COSM6210	p.L747_T751delinsS	c.2240_2251del12

Storage and Stability

QX700 ddPCR *EGFR* Exon 19 Deletions 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. Once thawed, the assay can be stored at 4°C for up to 2 weeks. The QX700 ddPCR Supermix for Probes remains liquid at −20°C and does not freeze solid.

Kit Contents

The duplex research 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 includes one tube of the 20x assay and eight tubes of QX700 ddPCR Supermix for Probes, both sufficient for 800 multiplex reactions. The following assays can be ordered individually for subsequent studies:

- 2235_2252>AAT (dHsaMDS391737271)
- 2235_2249del15 (dHsaMDV2010039)
- 2236_2250del15 (dHsaMDS542127747)
- 2236_2253del18 (dHsaMDS317319828)
- 2237_2254del18 (dHsaMDS399997555)
- 2237_2255>T (dHsaMDS823654281)
- 2237_2251del15 (dHsaMDS732829953)
- 2238_2248>GC (dHsaMDS742974650)
- 2238_2252>GCA (dHsaMDS435546404)
- 2238_2255del18 (dHsaMDS529140399)
- 2239_2253>CAA (dHsaMDS703176693)
- 2239_2251>C (dHsaMDV2516890)
- 2239_2258>CA (dHsaMDS196056967)
- 2239_2252>CA (dHsaMDS651224081)
- 2239_2256del18 (dHsaMDV2516758)
- 2239_2248TTAAGAGAAG>C (dHsaMDV2516748)
- 2239_2253del15 (dHsaMDV2516752)
- 2239_2247delTTAAGAGAA (dHsaMDS88236242)
- 2240_2251del12 (dHsaMDS509487982)
- 2240_2254del15 (dHsaMDS778667043)
- 2240_2257del18 (dHsaMDV2510546)

Reagents and Equipment

See Table 2 for required materials that are not included in the kit.

Table 2. 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
	• EcoRI restriction enzyme
	• RDG16 Cartridges, pack of 12 (#12025252)
	• Staticide SW12 Anti-Static Wipes (ACL, Inc.)
	• Kimtech Science Precision Wipes (Kimberly Clark, catalog #34155)

Protocol

Step 1: Restriction Enzyme Digestion 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. 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 sample DNA can be achieved by direct addition of restriction enzyme to the ddPCR mix
- 3 units of EcoRI per 5 µL Droplet Digital PCR is recommended
- Reactions can be set up at room temperature; no additional incubation time is required
- The addition of restriction enzyme buffers with a high salt content 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 EcoRI 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 3, without the restriction enzyme

Step 2: Reaction and Cartridge Setup

1. Thaw all components to room temperature. Mix thoroughly by vortexing the tube at maximum speed for 15 sec to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of the tubes.
2. Prepare samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix at room temperature for the number of reactions required according to the guidelines in Table 3. Assemble all required components except the sample and dispense equal aliquots into each reaction tube. As the final step, add the sample to each reaction tube.

Table 3. Reaction mix preparation.

Components	Volume per Reaction, µL	Final Concentration
QX700 ddPCR Supermix for Probes	1	1x
QX700 ddPCR *EGFR* Exon 19 Deletions Screening Assay	1	1x
EcoRI restriction enzyme*	Variable	3 U/reaction
Research DNA sample	Variable	Up to 132 ng per reaction
RNase-/DNase-free water	Variable	–
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 the QX700 Droplet Digital PCR System Instrument Guide (10000258608) and RDG16 Instructions for Use (10000171484) for sample and plate setup instructions.

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

QX700 ddPCR *EGFR* Exon 19 Deletions 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 *EGFR* Exon 19 Deletions 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 the QX700 ddPCR System, refer to the 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 thermo cycling program according to Table 4.

Table 4. Thermal cycling protocol.

Cycling Step	Temperature, °C	Time, sec	Cycles	Ramp Rate, °C/s
Enzyme activation	95	600	1	1
Denaturation	95	15	40	1
Annealing/ Extension	55	60	40	1

3. Define the reading program according to Table 5. 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 remain enabled for acquisition.

Table 5. Enabled channels with associated fluorophores and exposure time.

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

Assay File Setup

1. Under the Assay Structure section, ensure that the three channels listed in Table 6 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

Table 6. Assay structure details.

Dye Name	Channel	Accepted 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 the QX700 Droplet Digital PCR System Instrument Guide (10000258608).

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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 Lines 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. Move the individual threshold lines for FAM and HEX where there is clear separation between the negative and positive clusters. Click **Apply** under the Fluorescence Thresholds section. See Figure 1 for an example FAM/HEX 2D plot. The mutant target is detected in the FAM (blue) channel, and the wild-type target is detected in the HEX (teal) channel. The Cy5 (red) channel is not used but will show background fluorescence.
5. Navigate to the Setup heading under the POST PROCESSING tab.
6. Select **Mutant Allelic Fraction (MAF)** as the Post Processing Type.
7. Select the FAM population and click **Add population to processing** under Settings.
8. Modify the Reference to the HEX population as needed and click **APPLY**.
9. Navigate to the Results heading under the POST PROCESSING tab to view the calculated MAF (%) of each sample.

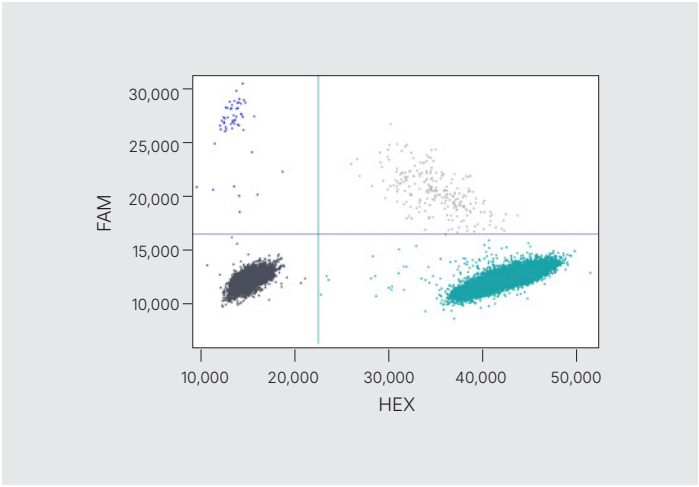

Fig. 1. Setting a threshold for ddPCR *EGFR* Exon 19 Deletions Screening Kit. Example FAM/HEX 2D plot for a mutation-positive control with a wild-type DNA background of 132 ng.

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Recommendations for Optimal Results

- Run at least one mutation-negative control with a wild-type DNA background 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
- 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 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/EGFR19del-700** for more information.

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