ddPCR™ EGFR Exon 19 Deletions Screening Kit

Catalog # 12002392

ddPCR™ EGFR Exon 19 Deletions Screening Kit, 200 x 20 μl reactions

For research purposes only.

Description
The ddPCR EGFR Exon 19 Deletions Screening Kit is designed to screen for 15 deletion mutations in Exon 19 of the EGFR gene (2236_2257del18, 2239_2247delTTAAGAGAA, 2239_2253del15, 2239_2256del18, 2239_2251>C, 2239_2253>CAA, 2238_2252>GCA, 2236_2250del15, 2235_2249AAT, 2235_2249del15, 2238_2252>GCA, 2238_2255del18, 2239_2247TTAAGAGAA>C, 2239_2253del15, 2239_2256del18, 2239_2251>C, 2239_2253>CAA, 2238_2252>GCA, 2238_2255del18, 2239_2253>CAA, 2238_2252>GCA, 2238_2255del18, 2239_2247TTAAGAGAA>C, 2239_2253del15, 2239_2256del18, 2239_2251>C, 2239_2253>CAA, 2238_2252>GCA, 2238_2255del18, 2239_2247TTAAGAGAA>C, 2239_2253del15, 2239_2256del18, and 2240_2257del18, plus wild-type). Other EGFR Exon 19 deletions present in this region may also be detected. EGFR Exon 19 deletions are commonly associated with melanoma, colorectal, and lung cancers. The multiplex assay is a 20x concentrated, ready-to-use primer-probe mix, and the ddPCR Supermix for Probes (No dUTP) is a 2x concentrated, ready-to-use supermix.

Storage and Stability
The ddPCR EGFR Exon 19 Deletions Screening Assay and ddPCR Supermix for Probes (No dUTP) included in the kit are stable through the expiration date printed on their labels when stored at -20°C and protected from light. Once thawed, the kit can be stored at 4°C for up to 2 weeks. Repeated freezing and thawing of the kit is not recommended.

Kit Contents
Each kit includes one tube of the 20x assay mix and two tubes of 2x ddPCR Supermix for Probes (No dUTP), both sufficient for 200 multiplex reactions. The following assays can be ordered individually for future studies:

- 2235_2252>AAT (Assay ID: dHsaMDS391737271), 2235_2249del15 (dHsaMVD2010039), 2236_2250del15 (dHsaMDS542127747), 2238_2252>GCA (dHsaMDS435546404), 2238_2255del18 (dHsaMDS29140399), 2239_2253>CAA (dHsaMDS703176693), 2239_2251>C (dHsaMVD2516890), 2239_2258>CA (dHsaMDS196056967), 2239_2252>CA (dHsaMDS651224081), 2239_2256del18 (dHsaMDS2516758), 2239_2253del15 (dHsaMDS71880807), 2239_2254>CA (dHsaMDS784236242), 2240_2252>GCA (dHsaMDS196056967), 2240_2257del18 (dHsaMDS196056967), and 2240_2257del18 (dHsaMDS784236242).

Required Equipment
- C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module (catalog #1851197)
- PX1™ PCR Plate Sealer (catalog #1814000)
- QX100 or QX200 Droplet Reader (catalog #1863003, respectively)
- Automated Droplet Generator (catalog #1863002 or 1864002, respectively)
- QX100 or QX200 Droplet Generator (catalog #1863003 or 1864003, respectively)
- QX100 or QX200 Droplet Generator (catalog #1863002 or 1864002, respectively)

Recommendations for Optimal Results
- Follow the general guidelines and recommendations in the Droplet Digital™ PCR Applications Guide and Rare Mutation Detection Best Practices Guidelines (bulletins 6407 and 6628)
- 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
- Run at least 1 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 (for example, a mix of 7 ng of mutant DNA in a background of 130 ng wild-type DNA)
- Run several no template control (NTC) wells

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 DNA samples: digestion directly in the ddPCR reaction during setup (recommended) or conventional digestion prior to Droplet Digital PCR.

Digestion Directly in ddPCR Reaction
- Efficient digestion of sample DNA can be achieved by direct addition of restriction enzyme to the ddPCR reaction
- 2–5 units of EcoR1 per ddPCR reaction are recommended
- Dilute the restriction enzyme using the recommended diluent buffer according to the manufacturer’s instructions, and then add 1 µl to the ddPCR reaction according to the guidelines in Table 1
- Reactions can be set up at room temperature; no additional incubation time is required

Quality Control
The ddPCR EGFR Exon 19 Deletions Screening Kit and ddPCR Supermix for Probes (No dUTP) are free of detectable DNase and RNase activities. Stringent specifications are maintained to ensure lot-to-lot consistency.
ddPCR™ EGFR Exon 19 Deletions Screening Kit

- The addition of restriction enzyme buffers with 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 reaction setup.
- Use 10–20 units of EcoR1 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 Droplet Digital PCR.
- Store digested DNA at −20°C or below.

Reaction Setup
1. Thaw all components to room temperature. Mix thoroughly by vortexing the tube to ensure homogeneity because a concentration gradient may form during −20°C storage. Centrifuge briefly to collect contents at the bottom of each tube and store protected from light.
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 needed according to the guidelines in Table 1. Assemble all required components except the sample, dispense equal aliquots into each reaction tube, and add the sample to each reaction tube as the final step.

Table 1. Preparation of the reaction mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per Reaction, µl</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x ddPCR Supermix for Probes (No dUTP)</td>
<td>10</td>
<td>1x</td>
</tr>
<tr>
<td>20x EGFR Exon 19 Deletions Screening Assay</td>
<td>1</td>
<td>1x</td>
</tr>
<tr>
<td>EcoR1 restriction enzyme, diluted*</td>
<td>1</td>
<td>2–5 U/reaction</td>
</tr>
<tr>
<td>DNA sample</td>
<td>Variable</td>
<td>50 fg–100 ng</td>
</tr>
<tr>
<td>RNase-/DNase-free water</td>
<td>Variable</td>
<td>–</td>
</tr>
<tr>
<td>Total Volume</td>
<td>20**</td>
<td>–</td>
</tr>
</tbody>
</table>

* This component should be replaced by water if digestion is performed prior to Droplet Digital PCR.
** For the Automated Droplet Generator, prepare 22 µl per well.

4. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow reaction tubes to equilibrate at room temperature for about 3 min.

5. Once the reaction mixtures are ready, load 20 µl of each reaction mix into a sample well of a DG8™ Cartridge (catalog #1864008) followed by 70 µl of Droplet Generation Oil for Probes (catalog #1863005) into the oil wells, according to the QX100 or QX200 Droplet Generator Instruction Manual (#10026322 or 10031907, respectively). For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (#10043138).

Thermal Cycling Conditions
1. After droplet generation with the QX100 or QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate or remove the plate containing ddPCR droplets from the Automated Droplet Generator. Seal the plate with the PX1 PCR Plate Sealer.
2. Proceed to thermal cycling (see protocol in Table 2) and subsequent reading of droplets in the QX100 or QX200 Droplet Reader.

Table 2. Cycling conditions for Bio-Rad’s C1000 Touch Thermal Cycler*.

<table>
<thead>
<tr>
<th>Cycling Step</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Ramp Rate</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activation</td>
<td>95</td>
<td>10 min</td>
<td>2°C/sec</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94</td>
<td>30 sec</td>
<td>–</td>
<td>40</td>
</tr>
<tr>
<td>Annealing/extension</td>
<td>55</td>
<td>1 min</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme deactivation</td>
<td>98</td>
<td>10 min</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Hold (optional)</td>
<td>4</td>
<td>Infinite</td>
<td>1°C/sec</td>
<td>1</td>
</tr>
</tbody>
</table>

* Use a heated lid set to 100°C and set the sample volume to 40 µl.

Data Acquisition and Analysis
1. After thermal cycling, place the sealed 96-well plate in the QX100 or QX200 Droplet Reader.
2. Open QuantaSoft™ Software to set up a new plate layout according to the experimental design. Refer to the QX100 or QX200 Droplet Reader and QuantaSoft Software Instruction Manual (catalog #10026321 or 10031906, respectively).
3. Under Setup, double click on a well in the plate layout to open the Well Editor dialog box.
4. Designate the sample name, experiment type, ddPCR Supermix for Probes (No dUTP) as the supermix type, target name(s), and target type(s): Ch1 for FAM and Ch2 for HEX or VIC.
5. Select Apply to load the wells and, when finished, select OK.
6. Once the plate layout is complete, select Run to begin the droplet reading process. Select the appropriate dye set and run options when prompted.
7. After data acquisition, select samples in the well selector under Analyze. Set a threshold for the ddPCR EGFR Exon 19 Deletions Screening Kit, as for any standard ddPCR mutation detection assay, by selecting the 2-D amplitude plot and setting the threshold lines close to the droplets that are positive for wild-type DNA (Figures 1A and 1C). Next, use the lasso tool to highlight any clusters that may not have been properly classified with the threshold lines (Figures 1B and 1D). For more detailed information about setting thresholds for mutation detection assays, please refer to the Rare Mutation Detection Best Practices Guidelines (bulletin 6628).
8. The concentration reported is copies/µl of the final 1x ddPCR reaction.
Fig. 1. Setting a threshold for the ddPCR EGFR Exon 19 Deletions Screening Kit. A and C, threshold lines set close to the droplets that are positive for wild-type DNA (green clusters); B and D, lasso tool selecting clusters not properly classified with the threshold lines (red circles).
ddPCR™ EGFR Exon 19 Deletions Screening Kit

FAM, HEX, and VIC are trademarks of Life Technologies Corporation.

Bio-Rad's thermal cyclers and real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

The QX100 and QX200 Droplet Digital PCR Systems and/or their use is covered by claims of U.S. patents, and/or pending U.S. and non-U.S. patent applications owned by or under license to Bio-Rad Laboratories, Inc. Purchase of the product includes a limited, non-transferable right under such intellectual property for use of the product for internal research purposes in the field of digital PCR only. No rights are granted for diagnostic uses. No rights are granted for use of the product for commercial applications of any kind, including but not limited to manufacturing, quality control, or commercial services, such as contract services or fee for services. Information concerning a license for such uses can be obtained from Bio-Rad Laboratories. It is the responsibility of the purchaser/end user to acquire any additional intellectual property rights that may be required.

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive, Hercules, CA 94547
1-800-424-6723

© 2016 Bio-Rad Laboratories, Inc.