

ddPCR™ *PIK3CA E542/E545/Q546/H1047* Screening Kit

Catalog #	Description
12003121	ddPCR™ <i>PIK3CA E542/E545/Q546/H1047</i> , 200 x 20 µl reactions

For research purposes only.

Description

The ddPCR *PIK3CA E542/E545/Q546/H1047* Screening Kit is designed to screen for one mutation in codon 542 (E542K), two mutations in codon 545 (E545K, E545G), one mutation in codon 546 (Q546K), and 2 mutations in codon 1047 (H1047L and H1047R). The *PIK3CA* Screening Kit generates two amplicons, one covering the E542/E545/Q546 codons and a second covering the H1047 codon. A HEX-labeled wild-type (WT) probe detects WT of the E542/E545/Q546 codons. The kit does not detect the WT of the H1047 codon. Fractional abundance of H1047L and H1047R is calculated using WT of the amplicon covering the E542/E545/Q546 codons. The expected phenotype of E542/E545/Q546 mutations is shown in Figure 1A and that of H1047 mutations is shown in Figure 2B. *PIK3CA* mutations are commonly associated with breast, colorectal, ovarian, 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 *PIK3CA E542/E545/Q546/H1047* 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 subsequent studies: E542K (Assay ID: dHsaMDV2010073), E545K (dHsaMDV2010075), E545G (dHsaMDV2510544), Q546K (dHsaMDS684842111), H1047L (dHsaMDV2010123), and H1047R (dHsaMDV2010077).

Required Equipment

- QX100™ or QX200™ Droplet Generator (catalog #1863002 or 1864002, respectively) or Automated Droplet Generator (catalog #1864101)
- QX100 or QX200 Droplet Reader (catalog #1863003 or 1864003, respectively)
- C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module (catalog #1851197)
- PX1™ PCR Plate Sealer (catalog #1814000)

Please refer to the QX100 or QX200 Instruction Manuals (#10026321 and 10026322 or 10031906 and 10031907, respectively) or the Automated Droplet Generator Instruction Manual (#10043138) for ordering information on consumables such as oils, cartridges, gaskets, plates, and seals.

Quality Control

The ddPCR *PIK3CA E542/E545/Q546/H1047* 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.

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 HaeIII 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
- 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 *HaeIII* 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

- 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.
- Prepare samples at the desired concentration before setting up the reaction mix.
- 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.

Component	Volume per Reaction, μ l	Final Concentration
2x ddPCR Supermix for Probes (No dUTP)	10	1x
20x <i>PIK3CA E542/E545/Q546/H1047</i> Screening Assay	1	1x
<i>HaeIII</i> restriction enzyme, diluted*	1	2–5 U/reaction
DNA sample	Variable	50 fg–100 ng
RNase-/DNase-free water	Variable	–
Total volume	20**	–

* Replace with water if digestion is performed prior to Droplet Digital PCR.

** For the Automated Droplet Generator, prepare 22 μ l per well.

- 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.
- Once the reaction mixtures are ready, load 20 μ l of each reaction mix into a sample well of a DG8™ Cartridge (catalog #1864008) followed by 70 μ l 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

- 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.
- 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*.

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

* Use a heated lid set to 105°C and set the sample volume to 40 μ l.

Data Acquisition and Analysis

- After thermal cycling, place the sealed 96-well plate in the QX100 or QX200 Droplet Reader.
- 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).
- Under Setup, double click on a well in the plate layout to open the Well Editor dialog box.
- 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.
- Select **Apply** to load the wells and, when finished, select **OK**.
- Once the plate layout is complete, select **Run** to begin the droplet reading process. Select the appropriate dye set and run options when prompted.
- After data acquisition, select samples in the well selector under Analyze. Set a threshold for the ddPCR *PIK3CA E542/E545/Q546/H1047* Screening Kit by selecting the 2-D amplitude plot and setting the threshold lines close to the droplets that are positive for wild-type DNA (Figure 2A). Next, use the lasso tool to highlight any clusters that may not have been properly classified with the threshold lines (Figure 2B). For more detailed information about setting thresholds for mutation detection assays, please refer to the Rare Mutation Detection Best Practices Guidelines (bulletin 6628).
- The concentration reported is copies/ μ l of the final 1x ddPCR reaction.

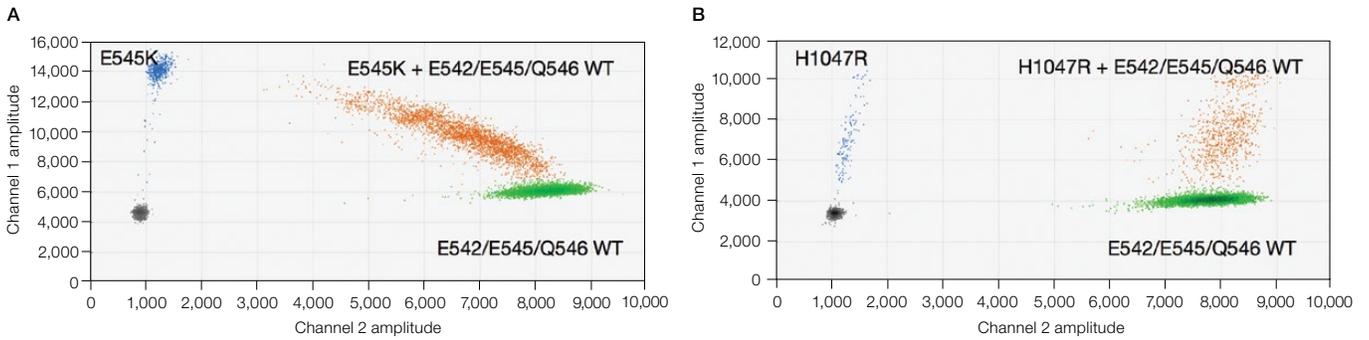


Fig. 1. 2-D plots with expected phenotypes. A, phenotype obtained with E542K, E545K, E545G, and Q546K; **B**, phenotype obtained with H1047R and H1047L.

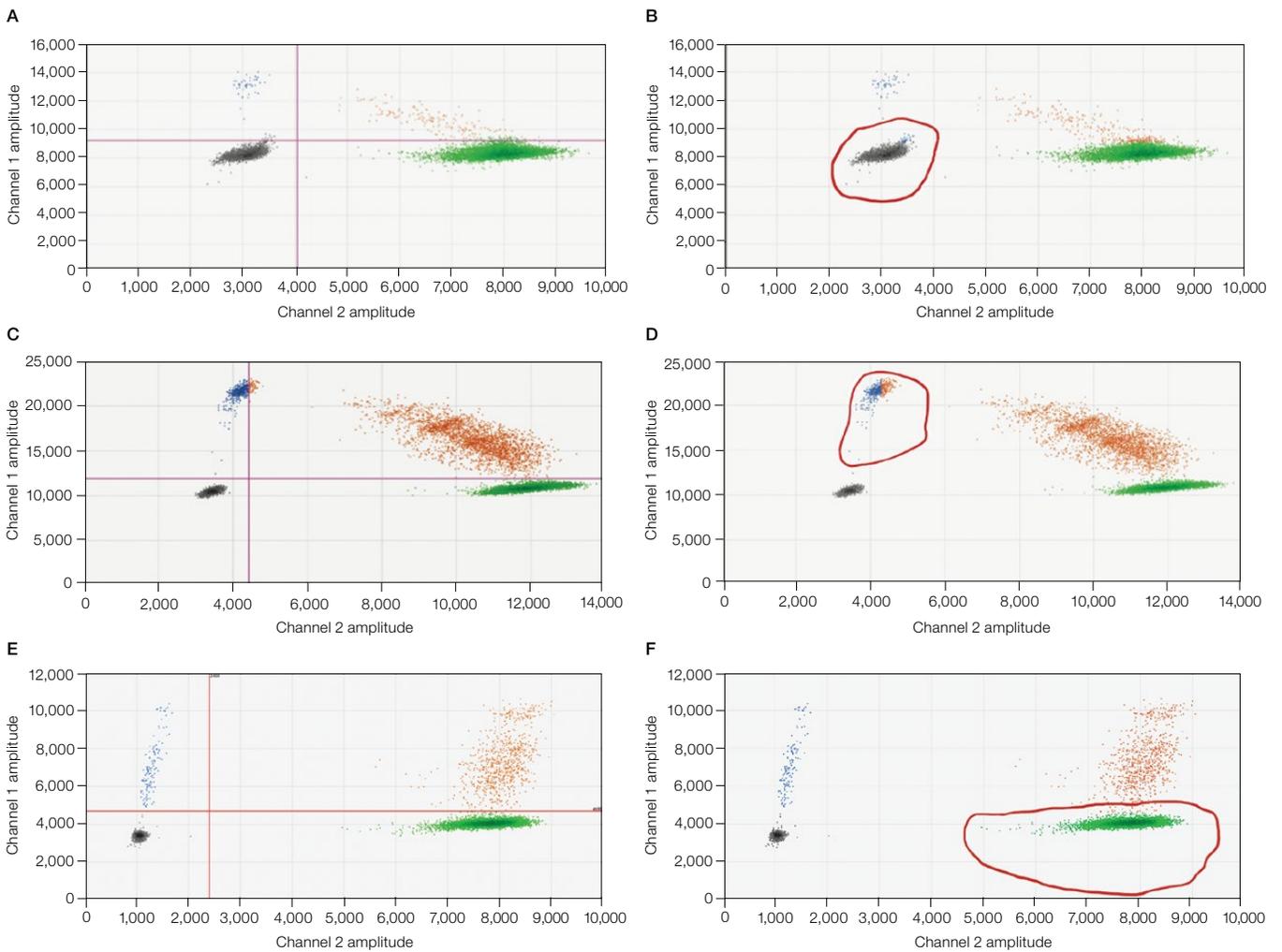


Fig. 2. Setting a threshold for the ddPCR PIK3CA E542/E545/Q546/H1047 Screening Kit. A, C, E, threshold lines set close to the droplets that are positive for wild-type DNA (green clusters); **B, D, F**, lasso tool selecting clusters not properly classified with the threshold lines (red circles).

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