Atila dPCR Lung Cancer Mutation Screening Kit

REF **ACPD-LN-100**

Instructions For Use

V2.0

March 2023

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IMPORTANT NOTICE

The instruction for use must be read carefully prior to use and followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from these instructions.

SYMBOLS

Symbol	Meaning
	Consult Instructions for Use
	Temperature Limitation
IVD	***In vitro* diagnostic medical device**
Σ	**Sufficient for <n> Tests**
	Use By
REF	**Catalogue Number**
LOT	**Batch Code**
EC REP	**Authorized Representative In the European Community**
	Manufacturer
CE	**CE mark**
LN-PM1	**LN Primer Mix 1**
LN-PM2	**LN Primer Mix 2**
LN-PM3	**LN Primer Mix 3**
LN-PM4	**LN Primer Mix 4**
LN-NC1	**LN Negative Control 1**
LN-NC2	**LN Negative Control 2**
LN-NC3	**LN Negative Control 3**
LN-NC4	**LN Negative Control 4**

INTENDED USE:

The Atila dPCR Lung Cancer Mutation Screening Kit allows quantification and screening for multiple Non-small cell lung cancer (NSCLC) mutations in four groups, provides sensitive and precise detection down to 0.5%, and allows screening of multiple samples in a rapid and cost-effective manner.

SUMMARY AND EXPLANATION OF THE TEST

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. It has become evident that somatic mutations and rearrangements in many different genes have been found in lung cancer cells, including EGFR, KRAS, HER2, MET and BRAF, etc. The status of genetic mutations can influence response to targeted therapy. Therefore, testing for these mutations and tailoring therapy accordingly are widely accepted as standard practice. The Atila digital PCR (dPCR) lung cancer mutation screening kit identifies 108 possible mutation sites in four groups in a rapid and cost-effective manner.

Mutation Coverage

Mutation Group	Gene				
Mutation Group 1	EGFR (A)	c.2154_2155GG>TT p.G719C	c.2155G>A p.G719S	c.2155G>T p.G719C	c.2156G>A p.G719D
		c.2156G>C p.G719A	c.2159C>T p.S720F	c.2161G>A p.G721S	c.2162G>C p.G721A
	EGFR (B)	c.2233_2247del15 p.K745_E749del	c.2234A>G p.K745R	c.2235_2246del12 p.E746_E749del	c.2235_2249del15 p.E746_A750del
		c.2235_2252del18 p.E746_T751del	c.2236G>A p.E746K	c.2236_2250del15 p.E746_A750del	c.2236_2253del18 p.E746_T751del
		c.2236_2256del21 p.E746_S752del	c.2237_2251del15 p.E746_T751>A	c.2237_2252>T p.E746_T751>V	c.2237_2254del18 p.E746_S752>A
		c.2237_2255>T p.E746_S752>V	c.2260A>G p.K754E	c.2238_2255del18 p.E746_S752>D	c.2239_2240TT>CC p.L747P
		c.2239_2247del9 p.L747_E749del	c.2239_2248>C p.L747_A750>P	c.2239_2251>C p.L747_T751>P	c.2239_2253del15 p.L747_T751del
		c.2239_2256del18 p.L747_S752del	c.2238_2252del15 p.L747_T751del	c.2240T>C p.L747S	c.2240_2251del12 p.L747_T751>S
		c.2240_2254del15 p.L747_T751del	c.2241_2244AAGA>CCCG p.L747_R748>FP	c.2240_2257del18 p.L747_P753>S	c.2248G>C p.A750P
		c.2252C>T p.T751I	c.2254T>C p.S752P	c.2255C>A p.S752Y	c.2257C>T p.P753S
	EGFR	c.2369C>T p.T790M	c.2573T>G p.L858R	c.2239_2257>T p.L747_P753>S	
Mutation Group 2	MET	c.3029C>T p.T1010I			
	BRAF	c.1799T>A p.V600E			
	KRAS (C)	c.43G>A p.G15S	c.40G>A p.V14I	c.39_40insGGC p.G13_V14insG	c.39C>G p.G13G
		c.39C>T p.G13G	c.39C>A p.G13G	c.38_39GC>TT p.G13V	c.38_39GC>TG p.G13V
		c.38_39GC>AT p.G13D	c.38_39GC>AA p.G13E	c.37_39GGC>CGT p.G13R	c.38G>A p.G13D
		c.38G>C p.G13A	c.38G>T p.G13V	c.37G>T p.G13C	c.37G>A p.G13S
		c.37G>C p.G13R	c.36_37insGGT p.G12_G13insG	c.36_37TG>AT p.G13C	c.36T>C p.G12G
		c.36T>A p.G12G	c.35_36GT>AC p.G12D	c.35_36GT>TC p.G12V	c.35_36GT>AA p.G12E
		c.34_36GGT>TGG p.G12W	c.24A>G p.V8V	c.35G>T p.G12V	c.35G>A p.G12D
		c.30_31insGGA p.G10_A11insG	c.34_35GG>TA p.G12Y	c.34_35GG>AT p.G12I	c.34_35GG>TT p.G12F
		c.34_35GG>CT p.G12L	c.34G>T p.G12C	c.34G>A p.G12S	c.34G>C p.G12R
		c.33_34insGGAGCT p.A11_G12insGA	c.32C>T p.A11V	c.31G>C p.A11P	
	KRAS	c.34_36GGT>TGC p.G12C	c.35G>C p.G12A		
Mutation Group 3	KRAS	c.38G>A p.G13D	c.34G>A p.G12S	c.35G>T p.G12V	
	EGFR	c.2303G>T p.S768I			
	HER2 (B)	c.2326G>A p.G776S	c.2326_2327insTTT p.G776>VC	c.2326_2327insTGT p.G776>VC	c.2327_2328insTCT p.G776_V777insL
		c.2327G>T p.G776V	c.2329G>T p.V777L	c.2329G>A p.V777M	c.2330T>C p.V777A
		c.2331_2332insTGTGGG p.V777_G778insCG	c.2333_2334insGGG p.G778_S779insG	c.2332_2333insGGCTCCCCA p.G778_S779insLPS	c.2335_2336ins9 p.S779_P780insVGS
		c.2340_2341ins9 p.P780_Y781insGSP			
	HER2	c.2324_2325ins12 p.A775_G776insYVMA			
Mutation Group 4	KRAS	c.37G>T p.G13C	c.38G>A p.G13D		
	EGFR	c.2582T>A p.L861Q	c.2236_2250del15(GAATTAAGAGAAGCA) p.E746_A750del		

KIT COMPONENTS

1. LN Primer Mix 1 (LN-PM1) 440 µL X 1 tube
2. LN Primer Mix 2 (LN-PM2) 440 µL X 1 tube
3. LN Primer Mix 3 (LN-PM3) 440 µL X 1 tube
4. LN Primer Mix 4 (LN-PM4) 440 µL X 1 tube
5. LN Negative Control 1 (LN-NC1) 100 µL X 1 tube
6. LN Negative Control 2 (LN-NC2) 100 µL X 1 tube
7. LN Negative Control 3 (LN-NC3) 100 µL X 1 tube
8. LN Negative Control 4 (LN-NC4) 100 µL X 1 tube
9. Instructions for Use 1 booklet

KIT STORAGE INFORMATION

All kit components should be stored at -20°C freezer for long time storage until expiration date. If properly stored, up to 6 freeze-thaw cycles are allowed for each kit component.

EQUIPMENTS & MATERIALS REQUIRED BUT NOT SUPPLIED

- Nuclease free H_2O
- 1.5mL microcentrifuge tube, Pipets, Pipet Tips, Reagent Trough
- ddPCR™ Supermix for Probes (No dUTP) (BioRad 1863023)
- Droplet Generation Oil for Probes (BioRad 1863005)
- ddPCR™ Droplet Reader Oil (BioRad 1863004)
- PCR Plate Heat Seal, Foil, Pieceable (BioRad 1814040)
- DG8™ Gaskets for QX200™/QX100™ Droplet Generator (BioRad 1863009)
- DG8™ Cartridges for QX200™ /QX100™ Droplet Generator (BioRad 1864008)
- ddPCR™ 96-Well Plates (BioRad 12001925)
- C1000 Touch™ Thermal Cycler with 96-Well (BioRad 1851196, 1851197)
- QX200 Droplet Digital PCR System (BioRad 1864001)
- PX1 PCR Plate Sealer (BioRad 1814000)
- QIAcuity™ Probe PCR Kit (1 ml) Cat. No. / ID: 250101
- QIAcuity™ Nanoplate 26k 24-well (10) Cat. No. / ID: 250001
- QIAcuity™ One, 5plex Device Cat. No. / ID: 911021
- cfDNA extraction and purification kit

WARNINGS AND PRECAUTIONS

General

- Handle all specimens as if they contain potentially infectious agents.
- Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- In order to avoid DNA contamination, the use of two dedicated areas within the laboratory is recommended for pre-amplification steps and post-amplification steps: A pre-amplification area is dedicated to processing samples and to adding processed samples and controls to the reaction tubes. All reagents, laboratory coats, pipettes, pipette tips, and equipment used in the

pre-amplification steps must remain in this area and not be moved to the post-amplification area. Do not bring amplification product into the pre-amplification area. The post-amplification area is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the post-amplification area must remain in this area and not be moved to the pre-amplification area.

- CHANGE GLOVES if they come in contact with specimen/reagent or appear to be wet, to avoid contaminating other specimens/reagents. Change gloves before leaving work area and upon entry into work area.
- Avoid contact of specimen or reagent with skin, eyes and mucous membranes. If any fluid comes into contact with skin or eyes, wash with water. If a spill of any fluid occurs, dilute with water before wiping dry, and then wipe several times with wet towels.
- Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution.

Specimen

- Whole blood specimens of 10mL collected in Streck Cell-Free DNA BCT are suggested.
- Follow standard procedures for storage/transportation of blood specimens and isolation of plasma.

Assay/Reagent

- Read the instructions in this package insert carefully before processing samples.
- Store the kit properly and do not use the kit after the indicated expiration date.
- Do not freeze-thaw more than 6 times even if the kits are properly stored.
- Avoid microbial contamination of reagents.
- Avoid cross-contamination of reagents from each other. Freshly mix different components right before experiments following the instruction.
- Do not interchange or mix the same kit component with different lot numbers.
- Avoid leakage of amplification product into the laboratory environment through the whole procedure. After the assay is done, discard reaction plates immediately into a sealable bag.

TEST PROCEDURE

Assay Setup on the BioRad QX200 Droplet Digital PCR System

- Thaw all components to room temperature. Mix thoroughly by vertexing the tube to ensure homogeneity. Centrifuge briefly to collect contents at the bottom of each tube and store protected from light.

- Purified blood cfDNA is used as template in the assay. Customer needs to ensure the quality of purified cfDNA when choosing cfDNA purification kit. Suggested cfDNA purification kit is Qiagen #55284, QIAamp MinElute ccfDNA Midi Kit (50). Suggested elution volume is 40uL. Quantify purified cfDNA with Qubit fluorometer.

- Prepare and label 4 sets of **N** x 1.5mL microcentrifuge tubes, **N** = number of samples + 2.

- Each sample requires four tubes of reactions. For each reaction, recommended input of cfDNA is about 1-5 ng. Add cfDNA to each of the four tubes and bring volume to 6.6 µL by adding nuclease free water. Prepare the reaction mixes according to the guidelines in the following table.

Component Well 1	Volume per reaction
2x ddPCR Supermix for Probes (No dUTP)	11 µl x **N**
LN Primer Mix 1	4.4 µl x **N**
Total Volume	15.4 µl x **N**

Component Well 2	Volume per reaction
2x ddPCR Supermix for Probes (No dUTP)	11 µl x **N**
LN Primer Mix 2	4.4 µl x **N**
Total Volume	15.4 µl x **N**

Component Well 3	Volume per reaction
2x ddPCR Supermix for Probes (No dUTP)	11 µl x **N**
LN Primer Mix 3	4.4 µl x **N**
Total Volume	15.4 µl x **N**

Component Well 4	Volume per reaction
2x ddPCR Supermix for Probes (No dUTP)	11 µl x **N**
LN Primer Mix 4	4.4 µl x **N**
Total Volume	15.4 µl x **N**

- For each sample, add 15.4 µL of component well 1 to tube 1, add 15.4 µL of component well 2 to tube 2, add 15.4 µL of component well 3 to tube 3, and add 15.4 µL of component well 4 to tube 4.

- NTC (blank) and Negative Control (provided in the kit) reactions are recommended. For NTC, add 6.6 µl nuclease free water. The suggested amount of negative control DNA is 2ng per reaction, add nuclease free water to bring volume to 6.6 µl.

- Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube.

- Follow manufacturer's manuals (QX200™ Droplet Generator Instruction Manual and QX200™ Droplet Reader and QuantaSoft™ Software Instruction Manual) to prepare ddPCR reactions. Briefly, insert the DG8 cartridge into the holder with the notch in the cartridge at the upper left of the holder. Each sample needs one cartridge and one Gasket. Gently transfer 20µL reaction mix into each of the 8 reaction wells (middle wells) on the cartridge. Pipet 70µL Droplet Generation Oil for Probes into each of the 8 oil wells (bottom wells) on the cartridge. Hook the gasket over the cartridge holder using the holes on both sides. Place the cartridge holder in the droplet generator and initiate droplet generation.

- When droplet generation is complete, remove the cartridge holder from the droplet generator. Remove and discard the gasket. Gently transfer droplets from top rows to PCR reaction plate.

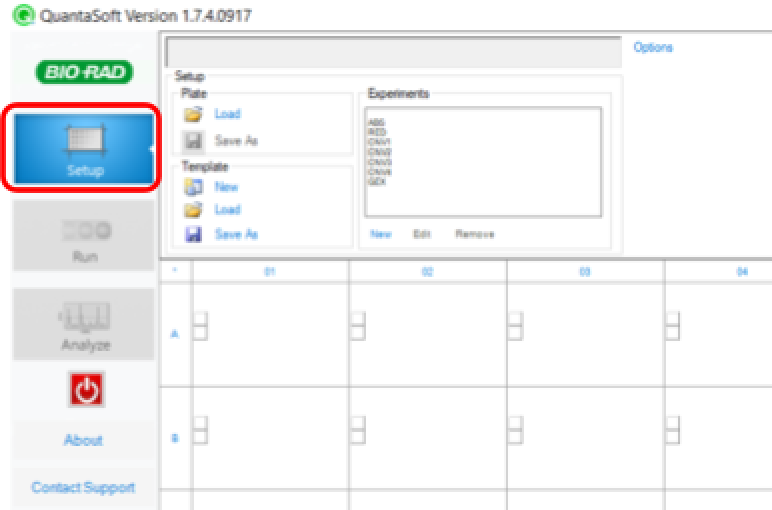
- Repeat the process for all samples. Seal the PCR plate with a pierceable foil plate seal immediately. Place the reaction plate in PCR machine and use the following program to complete PCR. Set the ramp rate for every step to 2°C/second.

Cycling Step	Temperature	Time	Ramp Rate
Enzyme activation	95 °C	10 min	2 °C /sec
Cycling × 60	95 °C	15 sec	2 °C /sec
	58 °C	50 sec	2 °C /sec
Enzyme deactivation	98 °C	10 min	2 °C /sec
Hold	4 °C	Infinite	2 °C /sec

- Cover the plate with aluminum foil and incubate the plate at room temperature for at least 2 hours, preferably overnight, before reading on the droplet reader. Droplets are stable for at least a week if stored at 4°C.

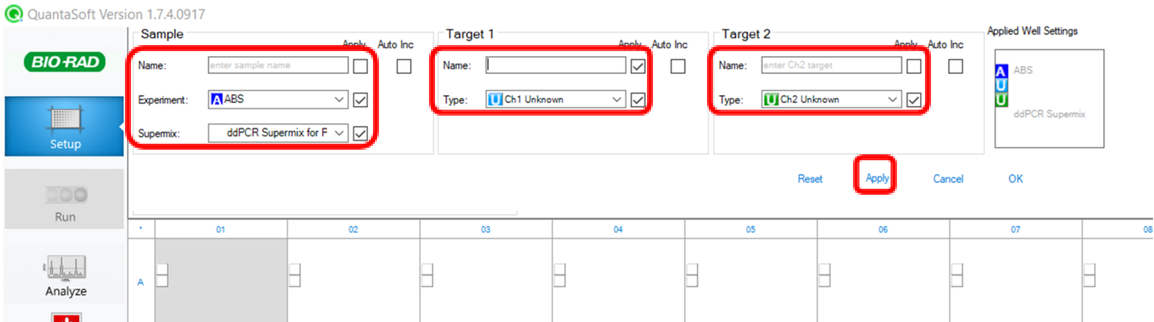
Data Collection on QX200 Droplet Reader

- Open QuantaSoft Software (Version 1.7.4.0917)
- On the main interface, click Setup.



- Double click on the selected wells.
- In Sample Tab:
  Name: Sample name
  Experiment: select ABS
  Supermix: select ddPCR Supermix For Probes (no dUTP)

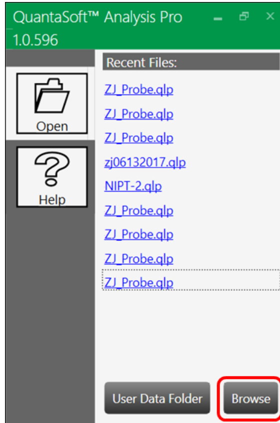
- In Target 1 Tab:
  Name: FAM Channel
  Type: Ch1 Unknown
- In Target 2 Tab:
  Name: HEX Channel
  Type: Ch2 Unknown
- Click Apply to complete setup.



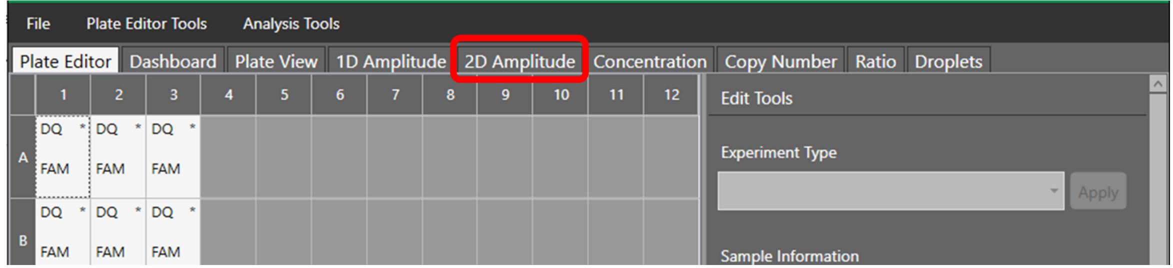
- Click Run. Save the template as instructed.
- In confirmation dialogue, select Columns for Acquire Data By option. Select FAM/HEX for Dye Set option. Click OK to start reading droplets. Data is automatically saved in the data/template_name_current date directory.

Data analysis with QuantaSoft Analysis Pro

- Open QuantaSoft Analysis Pro (QSAP is a free software downloadable on BioRad website). On the main interface, click Browse, open .qlp data file.



- Manual threshold setup: On the main interface, click 2D Amplitude tab.



- Select reaction wells on the bottom. The picture below shows how clusters are located on a 2D plot. EGFR(A) WT, and EGFR(B) WT should show up on Well 1. MET WT, KRAS(C) WT, and BRAF WT should show up on Well 2. EGFR WT and HER2(B) WT should show up on Well 3. KRAS WT and EGFT WT should show up on Well 4.

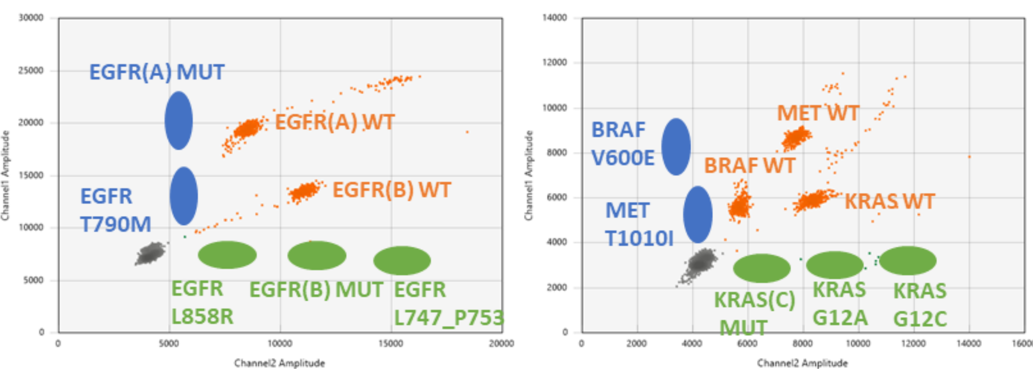


Fig. Well 1

Fig. Well 2

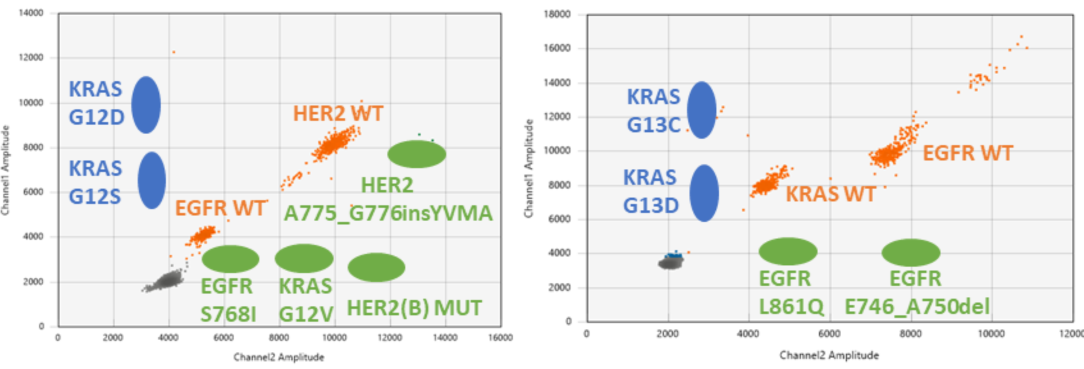
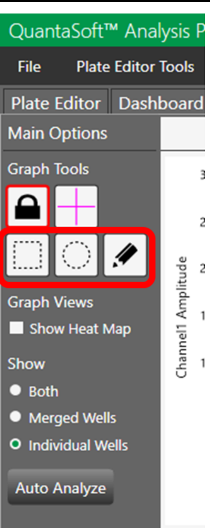


Fig. Well 3

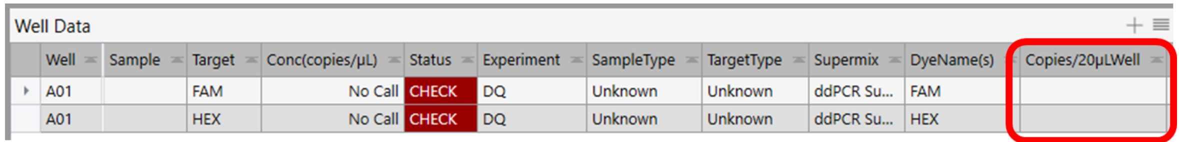
Fig. Well 4

- Copy number calculation: use **Threshold Cluster Mode** (lasso) to select all droplet groups and assign negative (Gray). Select target mutant droplets group and assign "FAM" (Blue). Meanwhile select the corresponding wildtype droplets group and assign "HEX" (Green). The mutants and their corresponding wildtype are listed in the following table. For EGFR T790M and EGFR L858R mutants in well #1, select all wildtype droplets group and assign "HEX" (Green). For KRAS G12D, KRAS G12S and KRAS G12V mutants in well #3, select all wildtype droplets group and assign "HEX" (Green).

Mutation Group	Mutant	Corresponding Wildtype Cluster
Mutation Group 1	EGFR A MUT	EGFR A WT
	EGFR L747_P753S	EGFR B WT
	EGFR B MUT	
	EGFR T790M	All WT
	EGFR L858R	
Mutation Group 2	BRAF V600E	BRAF WT
	MET T1010I	MET WT
	KRAS G12A	KRAS WT
	KRAS G12C	
	KRAS C MUT	
Mutation Group 3	EGFR S768I	EGFR WT
	HER2 A775_G776 insYVMA	HER2 WT
	HER2 B MUT	
	KRAS G12D	All WT
	KRAS G12S	
	KRAS G12V	
Mutation Group 4	KRAS G13C	KRAS WT
	KRAS G13D	
	EGFR L861Q	EGFR WT
	EGFR E746_A750del	



- The obtained copy number in the column “Copies/20µl” is the total copy number of the target in the reaction mix. Target “FAM” is the mutant copy number and target “HEX” is the wild type copy number.


Well Data

Well	Sample	Target	Conc(copies/µL)	Status	Experiment	SampleType	TargetType	Supermix	DyeName(s)	Copies/20µLWell
A01		FAM	No Call	CHECK	DQ	Unknown	Unknown	ddPCR Su...	FAM	
A01		HEX	No Call	CHECK	DQ	Unknown	Unknown	ddPCR Su...	HEX	

- Percentage of mutation in each group = copy number of mutant / (copy number of mutant + copy number of WT).
    - For EGFR T790M, EGFR L858R, KRAS G12D, KRAS G12S and KRAS G12V, Percentage of mutation = copy number of mutant / (copy number of WT / 2)

- For Example:
    - Use **Threshold Cluster Mode** select and set all droplets of the well as negative (gray).
    - Use **Threshold Cluster Mode** circle out one of the mutation droplets and set as FAM.
    - Read the number under Copies/20µl Well and get reading as **M**.
    - Use **Threshold Cluster Mode** circle out corresponding WT droplets and set as HEX.
    - Read the number under Copies/20µl Well and get reading as **W**.
    - The % of mutation = **M** / (**M**+**W**)
    - Repeat all steps for other mutations.

Assay Setup on QIAcuity Digital PCR System

- Thaw all components to room temperature. Mix thoroughly by vertexing the tube to ensure homogeneity. Centrifuge briefly to collect contents at the bottom of each tube and store protected from light.

- Purified blood cfDNA is used as template in the assay. Customer needs to ensure the quality of purified cfDNA when choosing cfDNA purification kit. Suggested cfDNA purification kit is Qiagen #55284, QIAamp MinElute ccfDNA Midi Kit (50). Suggested elution volume is 40uL. Quantify purified cfDNA with Qubit fluorometer.

- Prepare and label 2 sets of **N** x 1.5mL microcentrifuge tubes, **N** = number of samples + 2.

- Each sample requires two wells of reactions. For each reaction, recommended input of cfDNA is about 1-5 ng. Add cfDNA to each of the two tubes and bring volume to 24.2 µl by adding nuclease free water.

Component Group 1 and 2	Volume per reaction
QIAcuity™ probe PCR mix	11 x **N**
LN Primer Mix 1	4.4 µl x **N**
LN Primer Mix 2	4.4 µl x **N**
Total Volume	19.8 µl x **N**

Component Group 3 and 4	Volume per reaction
QIAcuity™ probe PCR mix	11 x **N**
LN Primer Mix 3	4.4 µl x **N**
LN Primer Mix 4	4.4 µl x **N**
Total Volume	19.8 µl x **N**

- NTC (blank) and Negative Control (provided in the kit) reactions are recommended. For NTC, add 24.2 µl nuclease free water. The suggested amount of negative control DNA is 2ng per reaction, add nuclease free water to bring volume to 24.2 µl.

- For each sample, NTC, and Negative Control, add 19.8 µl component group 1 and 2 prepared in the above table into reaction tube 1, add 19.8 µl component group 3 and 4 prepared in the above table into reaction tube 2.

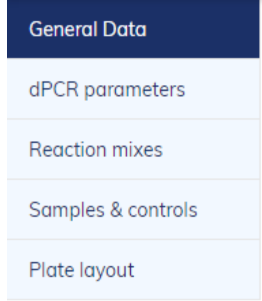
- Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube.

- Follow the manufacturer's manual (QIAcuity™ User Manual) to setup a digital PCR experiment on the QIAcuity™ instrument. The following steps and pictures are copied or modified from the mentioned manufacturer's manual and QIAcuity™ Software Suite.

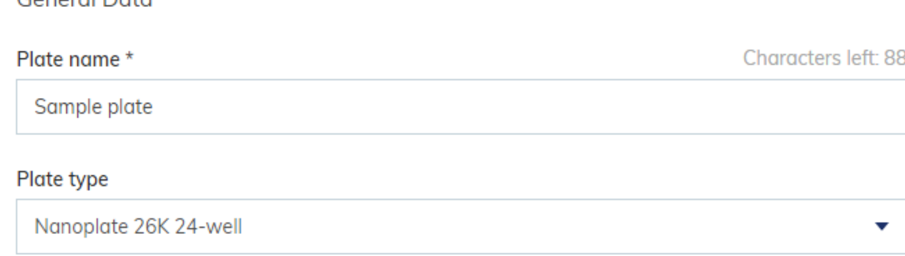
- Start the QIAcuity™ Software Suite. The default login is "admin" and "admin".

- Click on "New Plate".

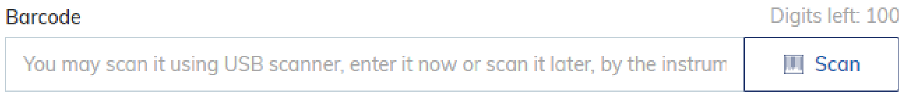
- In the tab "General Data".



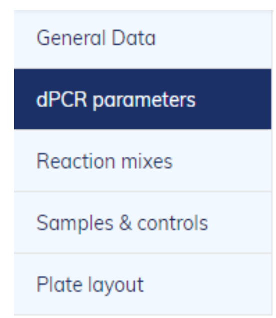
- Enter "Plate Name" and select "Nanoplate 26K 24-Well" in "Plate Type".



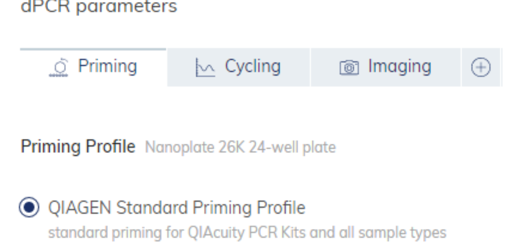
- Enter or scan the barcode of the plate in use.



- Go to the next tab "dPCR parameters".

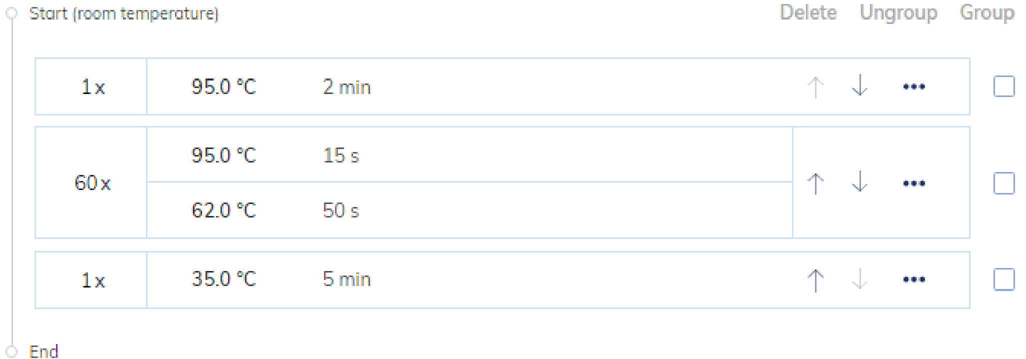


- Under the "Priming" tab, check "QIAGEN Standard Priming Profile".

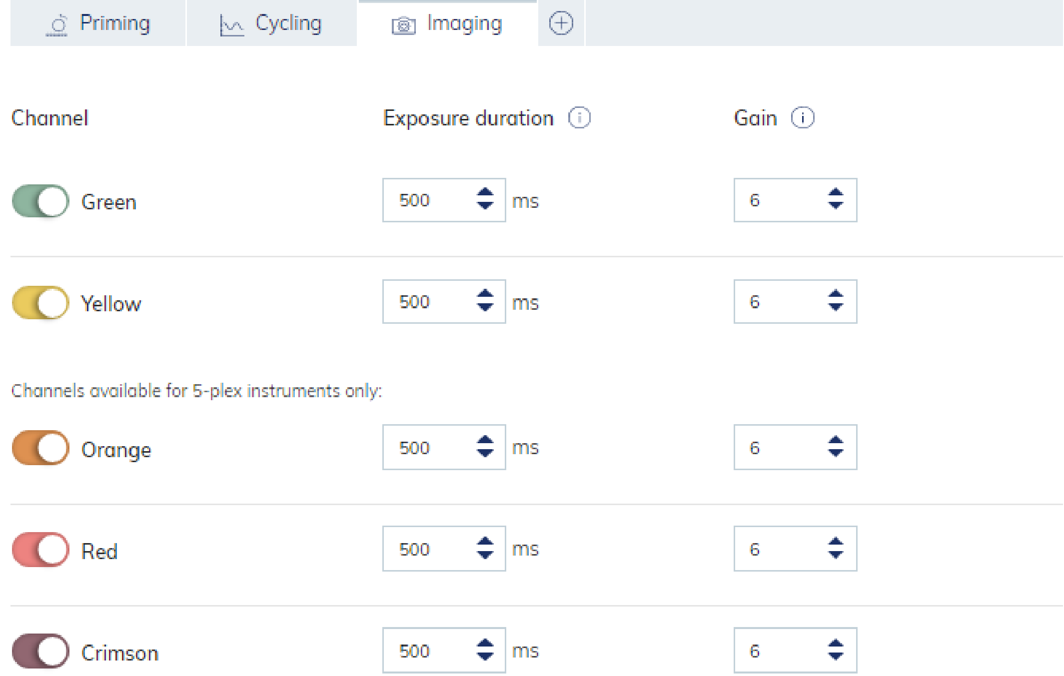


- Under the "Cycling" tab, set up the PCR program as the following table.

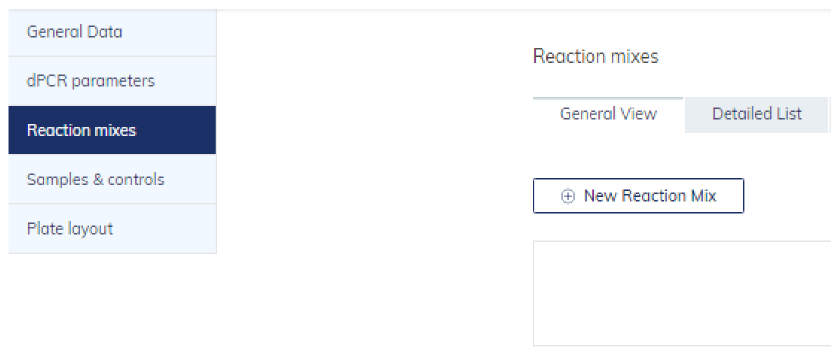
Cycle	Stage	Temp (°C)	Time
Hold	Polymerase activation	95	2 minutes
Cycle x 60	Denaturation	95	15 seconds
	Annealing	62	50 seconds
Hold	Storage	35	5 min or as needed



- Under the “Imaging” tab, enter the following values.

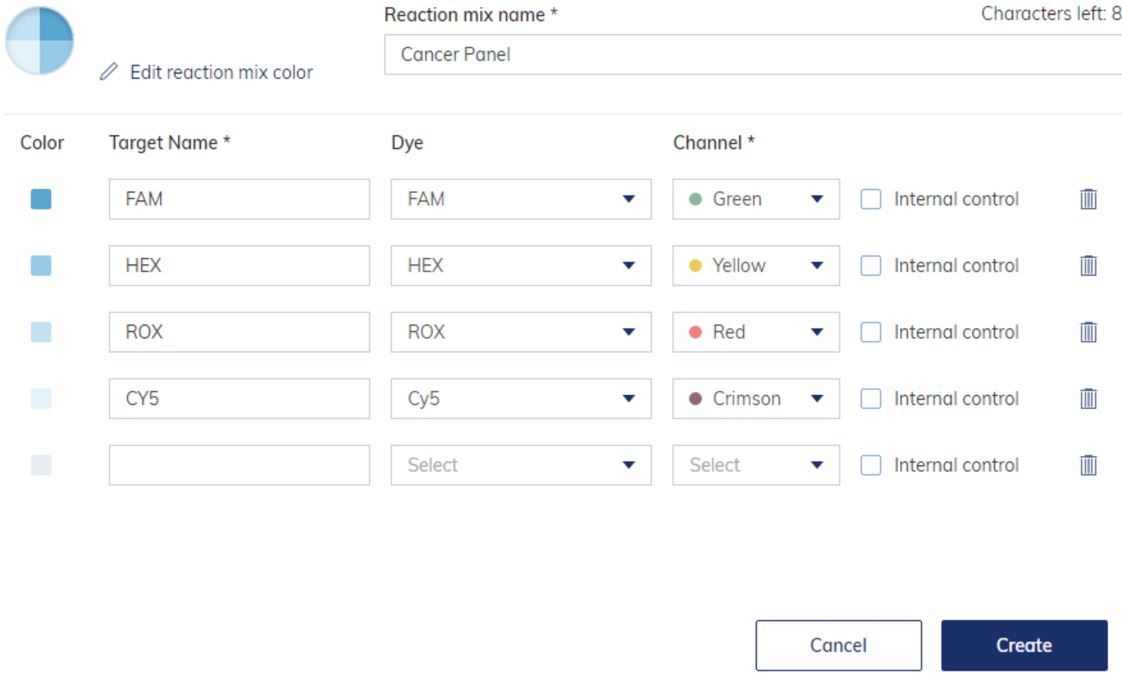


- Go to the next tab “Reaction mixes”. Click on “New Reaction Mix”.

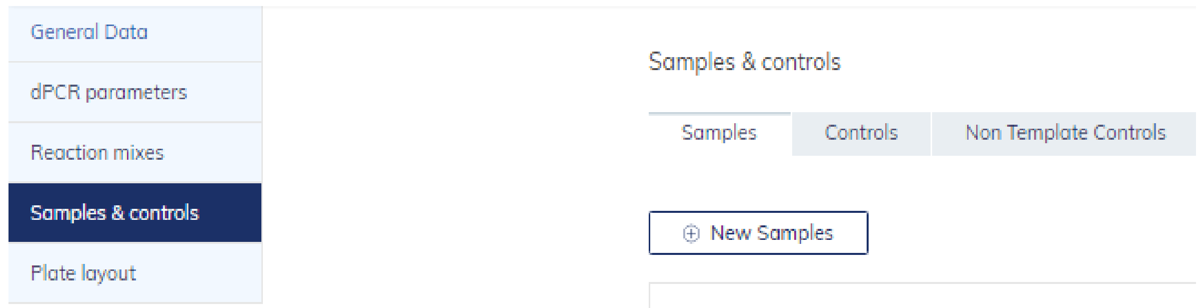


- Enter the “Reaction mix name”, “Target Name”, and select the “Dye” following the table and the picture below. Click on “Create”.

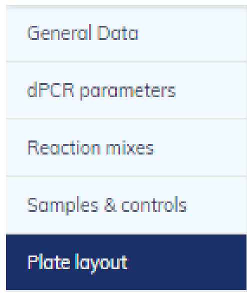
Target Name	Dye	Channel
FAM	FAM	Green
HEX	HEX	Yellow
ROX	ROX	RED
CY5	CY5	CRIMSON



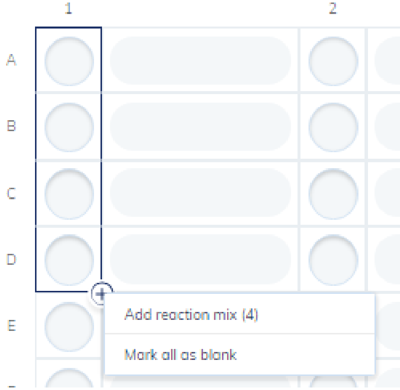
- Go to the next tab “Samples and controls”. Click on “New Samples” to enter sample information. Alternatively, sample information can be entered in the last tab “Plate layout”.

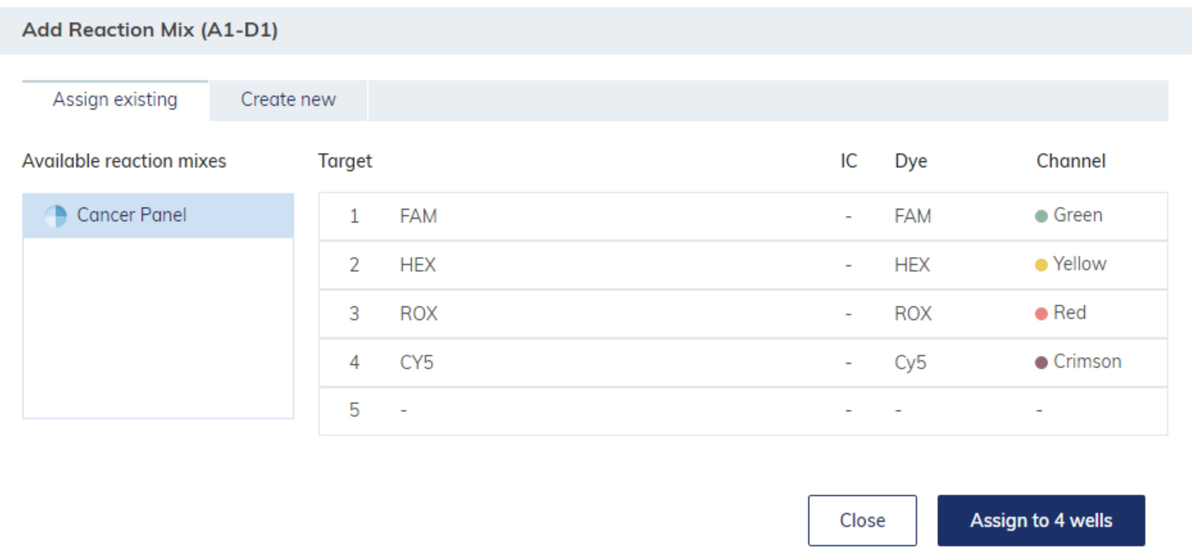


- Go to the next tab "Plate layout".

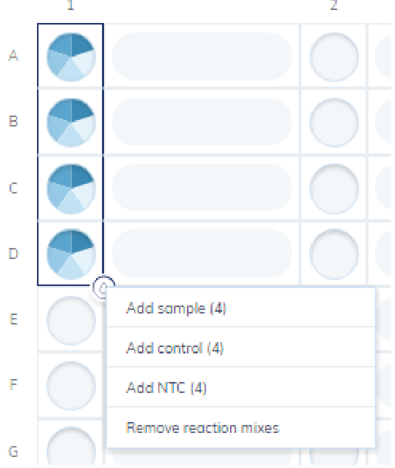


- Select the reaction wells, click on the "+", click on the "Add reaction mix", and assign the "Cancer Panel" to all reaction wells.





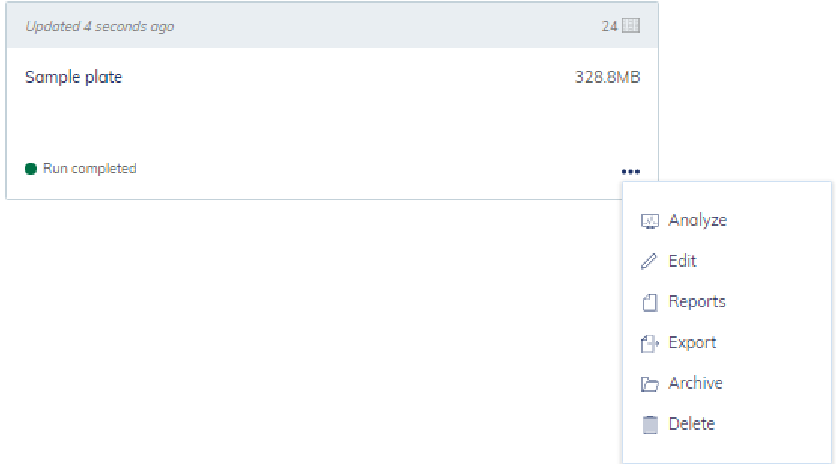
- Select the reactions wells for one sample, click on the “water drop” sign, and click on “Add sample”.



- Enter the sample name and click on “Create & assign to **X** wells”.
- Click on “Save Plate”.
- On the instrument, eject the plate holder, insert the plate, and close the plate holder.
- Wait for the instrument to read to barcode of the plate, make sure the plate name is correct, and click on the “Run” on the touch screen of the instrument.
- The experiment configuration of a past run can be saved as a plate template. The saved plate template can be imported when setting up a new run.

Data analysis with QIAcuity Software Suite

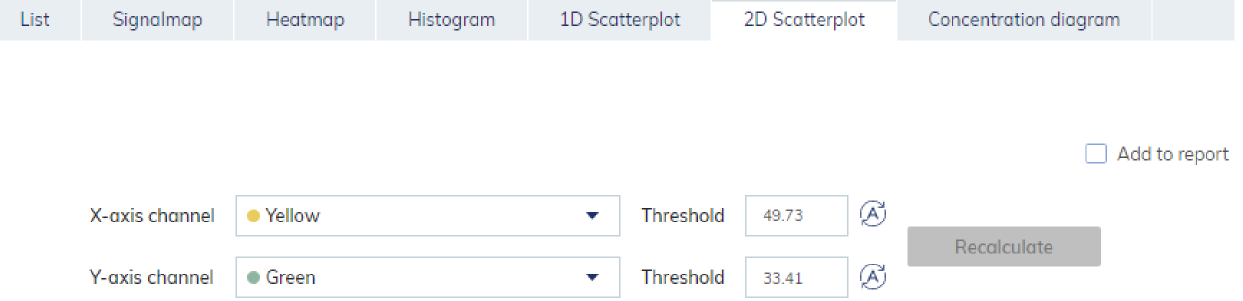
- Select the run file, click on the three dots, and click on “Analyze”.



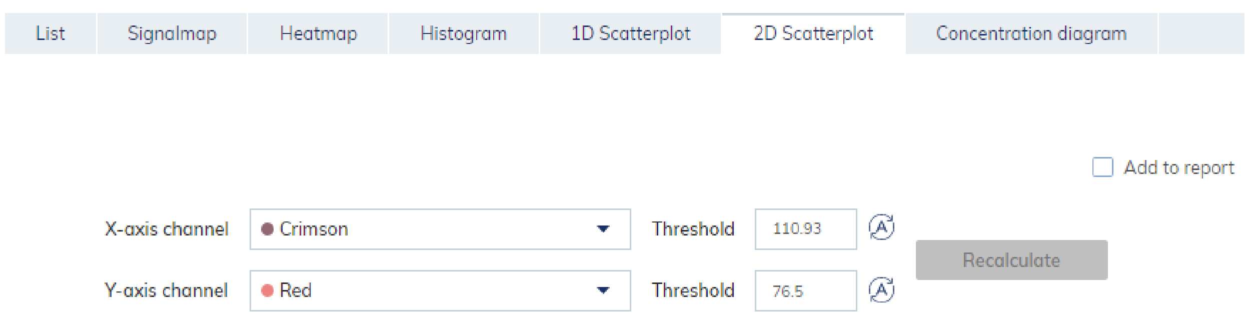
- Click on the reaction to be analyzed, click “Channel”, and click “Show results”.

- For each sample,
    - mutation group 1 is detected in the RED and CRIMSON channels of reaction 1.
    - mutation group 2 is detected in the GREEN and YELLOW channels of reaction 1.
    - mutation group 3 is detected in the RED and CRIMSON channels of reaction 2.
    - mutation group 4 is detected in the GREEN and YELLOW channels of reaction 2.

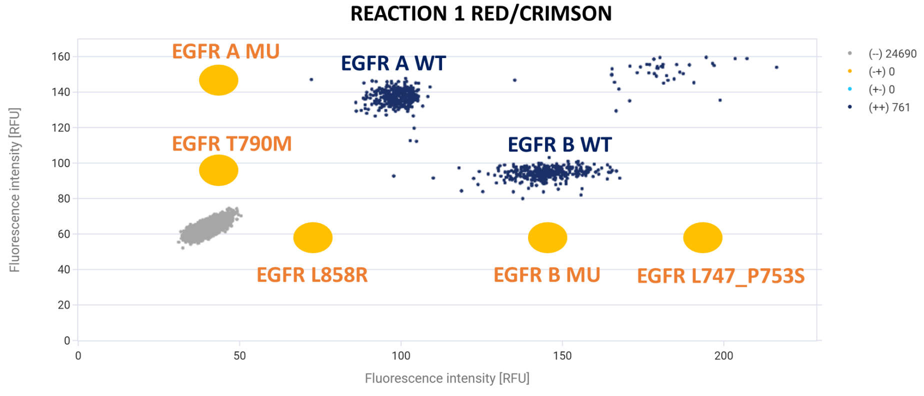
- For FAM and HEX channel analysis. Click on “2D Scatterplot”, select **“Yellow” as X-axis channel**, and select **“Green” as Y-axis channel**.

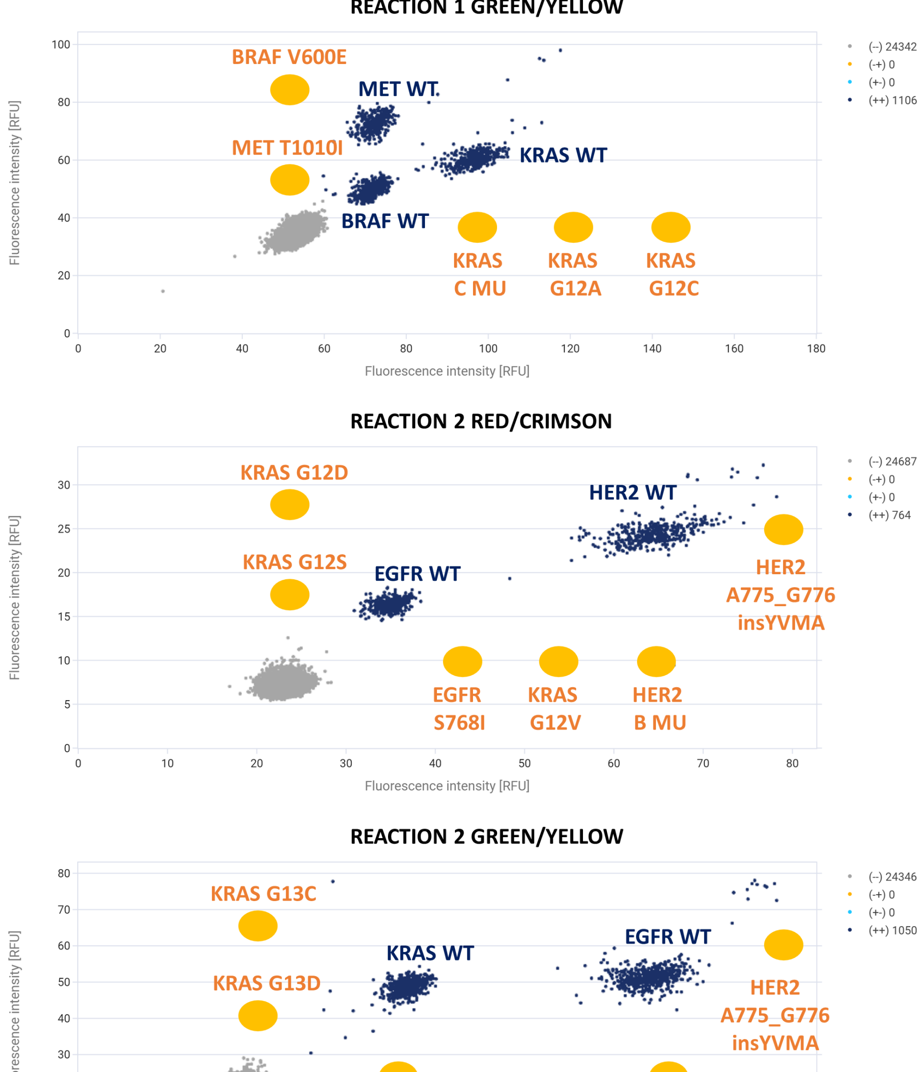


- For ROX and CY5 channel analysis. Click on “2D Scatterplot”, select **“Crimson” as X-axis channel**, and select **“Red” as Y-axis channel**.

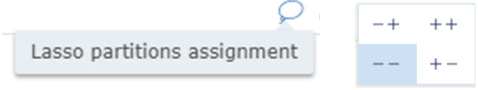


- NTC (blank) reactions should be generally free of any positive reactions.
- NC reactions should form only the WT clusters.
- The following plots show the locations of the WT and MU clusters.
  - For each sample, the WT clusters should be well separated, and their locations should match those of the NC reactions.

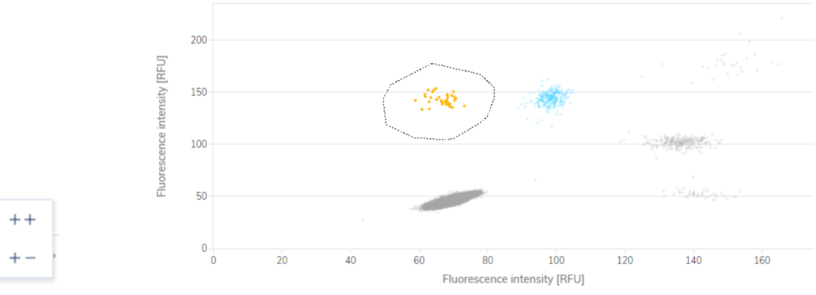




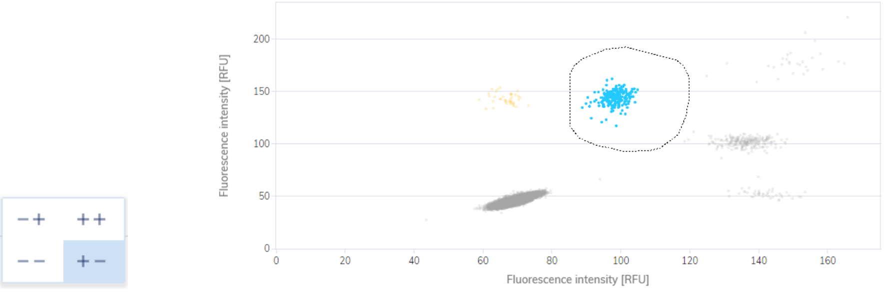
- Copy numbers of MU and WT are calculated in GREEN/YELLOW or RED/CRIMSON channels. The following steps should be repeated for each MU identified.
  - Use the "Lasso partitions assignment" to select all clusters and assign "- -".



  - Select target MU cluster and assign "- +".



- Select the corresponding WT cluster and assign “+ -” (see table below for MU and their corresponding WT clusters).



- Click “Recalculate” to update the positive cluster assignment.
- Click “List” to view the copy numbers in the column “Concentration copies/µL”.
- If the above is done in the GREEN/YELLOW channel
    - MU = number in GREEN channel
    - WT = number in YELLOW channel
- If the above is done in the RED/CRIMSON channel
    - MU = number in RED channel
    - WT = number in CRIMSON channel
- The MU % is calculated as
    - MU % = MU / (MU + WT) * 100%
    - For EGFR T790M, EGFR L858R, KRAS G12D, KRAS G12S and KRAS G12V, MU % = MU / (WT / 2) * 100%

- Repeat above steps for all MU identified.

Mutation Group	Mutant	Corresponding Wildtype Cluster
REACTION 1 RED CRISMSON	EGFR A MUT	EGFR A WT
	EGFR L747_P753S	EGFR B WT
	EGFR B MUT	
	EGFR T790M	All WT
	EGFR L858R	
REACTION 1 GREEN YELLOW	BRAF V600E	BRAF WT
	MET T1010I	MET WT
	KRAS G12A	KRAS WT
	KRAS G12C	
	KRAS C MUT	
REACTION 2 RED CRISMSON	EGFR S768I	EGFR WT
	HER2 A775_G776 insYVMA	HER2 WT
	HER2 B MUT	
	KRAS G12D	All WT
	KRAS G12S	
	KRAS G12V	
REACTION 2 GREEN YELLOW	KRAS G13C	KRAS WT
	KRAS G13D	
	EGFR L861Q	EGFR WT
	EGFR E746_A750del	

LIMITATIONS OF THE PROCEDURE

- The Atila dPCR Lung Cancer Mutation Screening Kit is a screening test. Decisions should not be based on the result of the screening alone.
- The Atila dPCR Lung Cancer Mutation Screening Kit applies to mutations listed in this IFU only. Other mutations are not tested.
- The Atila dPCR Lung Cancer Mutation Screening Kit requires peripheral whole blood samples.
- The Atila dPCR Lung Cancer Mutation Screening Kit requires a mutation fraction at least 0.5% to reach optimal performance.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity and Specificity

The analytical sensitivity of Atila dPCR Lung Cancer Mutation Screening Kit was determined in Limit of Detection (LoD) studies using the C1000 Touch™ Thermal Cycler with 96-Well (BioRad 1851196, 1851197) and QX200 Droplet Digital PCR System (BioRad 1864001). The analytical sensitivity was tested on artificial DNA samples containing 0.5% mutations and 99.5% healthy human genomic DNA. The

artificial DNA samples were prepared by diluting artificial DNA containing mutations into healthy human genomic DNA (Millipore Cat# 69237). The analytical specificity of Atila dPCR Lung Cancer Mutation Screening Kit was tested on the healthy human genomic DNA (Millipore Cat# 69237). The analytical sensitivity and specificity of the test are listed in Table 1 and 2, respectively.

Table 1, Analytical Sensitivity of Atila dPCR Lung Cancer Mutation Screening Kit

Sample Type	Target Mutations	Concentration	No. of replicates	No. of Positive Results	Percentage of Detection
Positive samples	EGFR G719S (A MUT)	0.5%	20	20	100%
	EGFR T790M	0.5%	20	20	100%
	EGFR L858R	0.5%	20	20	100%
	EGFR E746_A750del (B MUT)	0.5%	20	20	100%
	EGFR L747-P753	0.5%	20	20	100%
	BRAF V600E	0.5%	20	20	100%
	MET T1010I	0.5%	20	20	100%
	KRAS G12D (C MUT)	0.5%	20	20	100%
	KRAS G12A	0.5%	20	20	100%
	KRAS G12C	0.5%	20	20	100%
	KRAS G12D	0.5%	20	20	100%
	KRAS G12S	0.5%	20	20	100%
	EGFR S768I	0.5%	20	20	100%
	KRAS G12V	0.5%	20	20	100%
	HER2 p.P780_Y781insGSP (B MUT)	0.5%	20	20	100%
	HER2 A775_G776insYVMA	0.5%	20	20	100%
	KRAS G13C	0.5%	20	20	100%
	KRAS G13D	0.5%	20	20	100%
	EGFR L861Q	0.5%	20	20	100%
	EGFR E746_A750del	0.5%	20	20	100%

Table 2, Analytical Specificity of Atila dPCR Lung Cancer Mutation Screening Kit

Sample Type	Target Mutations	Concentration	No. of replicates	No. of Positive Results	Percentage of Detection
Negative samples	EGFR A MUT	0%	20	0	0%
	EGFR T790M	0%	20	0	0%
	EGFR L858R	0%	20	0	0%
	EGFR B MUT	0%	20	0	0%
	EGFR L747-P753S	0%	20	0	0%
	BRAF V600E	0%	20	0	0%
	MET T1010I	0%	20	0	0%
	KRAS C MUT	0%	20	0	0%
	KRAS G12A	0%	20	0	0%
	KRAS G12C	0%	20	0	0%
	KRAS G12D	0%	20	0	0%

	KRAS G12S	0%	20	0	0%
	EGFR S768I	0%	20	0	0%
	KRAS G12V	0%	20	0	0%
	HER2 B MUT	0%	20	0	0%
	HER2 A775_G776insYVMA	0%	20	0	0%
	KRAS G13C	0%	20	0	0%
	KRAS G13D	0%	20	0	0%
	EGFR L861Q	0%	20	0	0%
	EGFR E746_A750del	0%	20	0	0%

Interfering Substances:

Refer to BioRad Instruction Manuals for QX200 Droplet Reader, ddPCR Supermix for Probes (No dUTP) and QIAGEN Instruction Manuals for QIAcuity Digital PCR System for potential interfering substances to dPCR reactions.

Follow standard procedures to ensure the proper storage and transportation conditions of blood specimens.

Clinical Performance

Clinical ctDNA and FFPE DNA samples were tested with Atila dPCR Lung Cancer Mutation Screening Kit according to the instructions for use. Sample results for the Atila test were compared to the results of the NGS sequencing results. Results are summarized in Table 3 below.

Table 3, Clinical performance of Atila dPCR Lung Cancer Mutation Screening Kit				
Sample Set 1 KRAS C-1 MUT		**Comparator results**		
		Detected	**Not Detected**	**Total**
Atila results	**Detected**	8	0	8
	Not Detected	0	16	16
	Total	8	16	24
Sample Set 2 EGFR T790M		**Comparator results**		
		Detected	**Not Detected**	**Total**
Atila results	**Detected**	3	0	3
	Not Detected	0	4	4
	Total	3	4	7
Sample Set 2 EGFR L858R		**Comparator results**		
		Detected	**Not Detected**	**Total**
Atila results	**Detected**	3	0	3
	Not Detected	0	4	4
	Total	3	4	7
Sample Set 2 EGFR B MUT		**Comparator results**		
		Detected	**Not Detected**	**Total**

Atila results	Detected	2	0	2
	Not Detected	0	5	5
	Total	23	5	7

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