

EGFR 6-color Crystal Digital PCR® Kit [Nio™+]

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures.

For use by qualified personnel trained on the Nio™+.

Product Name

EGFR 6-color Crystal Digital PCR® Kit (R30006)

Number of reactions: 192 reactions (Ruby Chip)

Samples: cell-free DNA and FFPE genomic DNA

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Intended Use

The *EGFR* 6-color Crystal Digital PCR® Kit is intended for the detection and quantification of 32 somatic *EGFR* mutations in exons 18, 19, 20 and 21 associated with non-small cell lung cancer (NSCLC) on Nio™+ with the Ruby Chip.

The *EGFR* 6-color Crystal Digital PCR® Kit can be used on cell-free DNA (cfDNA) extracted from plasma and genomic DNA isolated from FFPE tissue sections.

Please note:

- The extraction method used and sample purity might have an influence on sample compatibility.
- **Individual sample-type and extraction method compatibilities for digital PCR applications may require a dedicated assay validation by the end user.**
- Compatible with Ruby Chip consumables on the Nio™+.

Table 1. Mutations Table

Exon	Mutation	Base changes	Cosmic ID
Exon 18	p.G719A	c.2156G>C	COSM6239
	p.G719C	c.2155G>T	COSM6253
	p.G719S	c.2155G>A	COSM6252
Exon 19	Exon 19 Deletions = All deletions between the bases c.2235 to c.2250 including:		
	p.E746-A750del	c.2236_2250del	COSM6225
	p.E746_T751del	c.2236_2253del	COSM12728
	p.E746-T751delinsA	c.2237_2251del	COSM12678
	p.E746_T751delinsI	c.2235_2252delinsAAT	COSM13551
	p.E746_S752delinsA	c.2237_2254del	COSM12367
	p.E746_S752delinsD	c.2238_2255del	COSM6220
	p.E746-S752delinsV	c.2237_2255delinsT	COSM12384
	p.E746-P753delinsVS	c.2237_2257delinsTCT	COSM18427
	p.L747-E749del	c.2239_2247del	COSM6218
	p.L747-A750delinsS	c.2240_2248del	COSM4170221
	p.L747-A750delinsP	c.2239_2248delinsC	COSM12382
	p.L747-A750delinsP	c.2238_2248delinsGC	COSM12422
	p.L747-T751del	c.2240_2254del	COSM12369
	p.L747-T751delinsP	c.2239_2251delinsC	COSM12383
	p.L747-T751delinsQ	c.2238_2252delinsGCA	COSM12419
	p.L747-A751delinsS	c.2240_2251del	COSM6210
p.L747-S752del	c.2239_2256del	COSM6255	
p.L747-P753delinsS	c.2240_2257del	COSM12370	
p.L747-P753delinsQ	c.2239_2258delinsCA	COSM12387	
Exon 20	p.T790M	c.2369C>T	COSM6240
	p.C797S	c.2389T>A	COSM6493937
	p.C797S	c.2390G>C	COSM5945664
	Exon 20 insertions = All insertions between the bases c. 2310 to c. 2320 including:		
	p.D770_N771insG	c.2310_2311insGGT	COSM12378
	p.H773-V774insH	c.2319-2320insCAC	COSM12377
	p.H773-V774insPH	c.2319-2320insCCCCAC	COSM28944
	p.H773-V774insAH	c.2319-2320ins-CCCACG	COSM1238028
	p.H773-V774insNPH	c.2319-2320insAAACCCCAC	COSM18491
	Exon 21	p.L858R	c.2573T>G
p.L861Q		c.2582T>A	COSM6213

The *EGFR* 6-color Crystal Digital PCR® Kit enables highly multiplexed DNA analysis on the Nio™+. It uses TaqMan® probe technology to detect eight-point mutations, as well as twenty-four Exon 19 deletion and Exon 20 insertion alterations through the use of drop-off detection (Figure 1). A drop-off digital PCR assay is the simplified detection of numerous proximal genetic lesions (including deletions, insertions and nucleotide substitutions) within a short genomic interval (refer to technical note “[How to quantify Drop-Off digital PCR Assays with Crystal Miner](#)”).

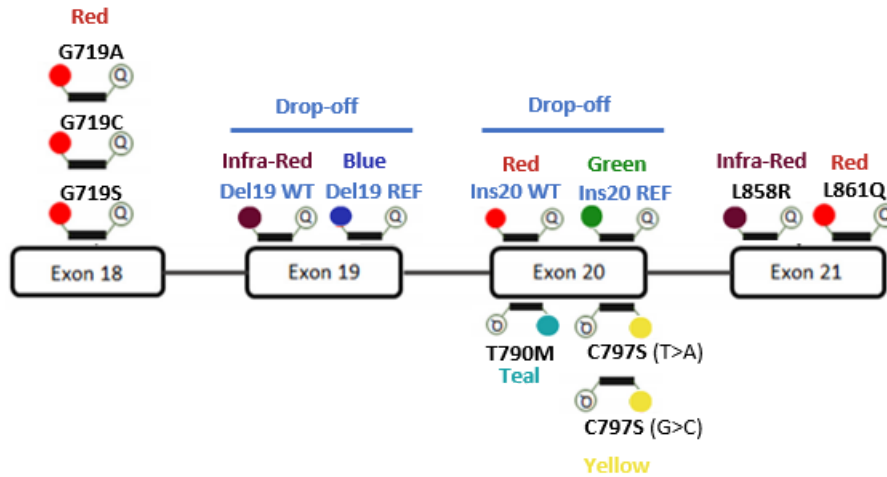


Figure 1. *EGFR* 6-color Crystal Digital PCR® Kit detection design.

Resources

Documents referenced in this Instructions for Use (IFU) can be accessed here:

<https://www.stillatechnologies.com/digital-pcr/naica-system-support/technical-resources/>

➤ Before using the *EGFR* 6-color Crystal Digital PCR® Kit for the first time:

Partitioning, PCR, and Image Acquisition steps:

Download the **.nioprotocol** and **.nioassay** files of the *EGFR* 6-color Crystal Digital PCR® Kit from the technical resources section of the Stilla® website on a USB flashdrive to import it to the Nio™+. Please refer to Nio™+ User Manual for details concerning Nio™+ use.

Interpretation of sample results step:

Request the **Certificate of Compliance** (CoC) of the *EGFR* 6-color Crystal Digital PCR® Kit from the Technical Support Department. The CoC contains the accepted concentration range for all targets in the positive control. As it is a batch number-related information, this step should be repeated for each new batch of *EGFR* 6-color Crystal Digital PCR® Kit.

Composition

The *EGFR* 6-color Crystal Digital PCR® Kit is packaged in a sealed box containing a total of six tubes:

Component Name	Cap Color	Initial Concentration	Volume	Description
Buffer A	Blue ●	10X	140 µL	naica® multiplex PCR MIX Buffer A
Buffer B	Red ●	50X	50 µL	naica® multiplex PCR MIX Buffer B
Buffer C	Yellow ●	8X	175 µL	Ready to use primers and TaqMan® probes
Positive Control	Purple ●	5X	100 µL	Synthetic mutated DNA solution (concentrations defined in CoC)
Negative Control	White ○	5X	100 µL	Nuclease-free water for no template control chambers, referred in the assay as “Neg Ctl”.
Nuclease-Free Water	Clear	N/A*	1000 µL	Nuclease-free water for volume adjustment



*N/A: not applicable

Materials Required but Not Provided

- Nio™+
- Standard consumables and equipment for PCR reaction mix preparation:
 - PCR-compliant reaction tubes
 - PCR-compliant 8-well strip tubes (*optional*)
 - Centrifuge for microcentrifuge tubes (~700xg)
 - Laboratory mixer - Vortex
 - Micropipettes (from 1 to 200 µL)
 - Micropipette tips
- Ruby Chip consumables (Reference C16011).
- Antistatic wetted wipes (ACL Staticide®, Reference: SW12).
→ The specific product can be ordered from Stilla® as a spare part (Part number H10000.472) or directly from the supplier Digi-Key using the reference ST1059-ND. [SW12 ACL Staticide Inc | Anti-Static, ESD, Clean Room Products | DigiKey.](#)
- Precision Wipes (Kimtech™ Science, Reference: 7552, 1 ply, 213x114 mm) can be ordered from standard laboratory suppliers.


Storage

Immediately upon reception, inspect package integrity and ensure the correct storage of the *EGFR* 6-color Crystal Digital PCR® Kit at the indicated storage temperatures. In case of doubt on the integrity or correct storage of the *EGFR* 6-color Crystal Digital PCR® Kit upon reception, do not use the kit and contact Technical Support.

- All components of the *EGFR* 6-color Crystal Digital PCR® Kit must be stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in the original tubes until the expiration date indicated on the respective packaging. Do not aliquot in alternative tubes.
-  Store tubes protected from light sources. It is recommended to store all tubes in the provided cardboard box.
-  Store tubes at all times in an upright position. It is recommended to store all tubes in the provided cardboard box.
- The *EGFR* 6-color Crystal Digital PCR® Kit can be thawed up to 10 times without observable deviations in performance and shelf-life.
- General consideration for reagent storage: all tube caps should be well-closed before stocking.

Under these conditions, the *EGFR* 6-color Crystal Digital PCR® Kit is stable until the expiration date indicated on the external packaging label.

Conditions for Use

- Operate the *EGFR* 6-color Crystal Digital PCR® Kit at a temperature ranging from $+20^{\circ}\text{C}$ to $+25^{\circ}\text{C}$.
- Ensure to thaw completely each *EGFR* 6-color Crystal Digital PCR® Kit reagent prior to reaction mix preparation.
-  Before each use, vortex each component thoroughly at maximum speed for 10 seconds and briefly centrifuge to collect the liquid at the bottom of the tube.
- Discard all *EGFR* 6-color Crystal Digital PCR® Kit reagent components as soon as one reagent component is empty.
- Discard all *EGFR* 6-color Crystal Digital PCR® Kit reagent components as soon as one reagent component is expired. The expiration date of the limiting component is indicated on the external packaging.
- Never combine *EGFR* 6-color Crystal Digital PCR® Kit reagent components from different *EGFR* 6-color Crystal Digital PCR® Kit boxes.

Assay Protocol

It is recommended to quantify the DNA concentrations of the samples before performing Crystal Digital PCR®. The *EGFR* 6-color Crystal Digital PCR® Kit is compatible with a range of DNA concentrations. However, users must not exceed 40 ng of DNA per reaction for optimal performance.

It is recommended to perform a minimum of one full Ruby Chip with one positive and one negative control included. For a full Ruby Chip run, it is recommended to add a minimum of two positive control chambers and one negative control chamber, irrespective of the number of clinical samples analyzed (from 1 to 45 cfDNA or FFPE samples).

*Note: Do not perform an overnight run with the *EGFR* 6-color Crystal Digital PCR® Kit.*

Step 1. Reaction Preparation

1. Thaw completely each *EGFR* 6-color Crystal Digital PCR® Kit reagent (equilibrate to room temperature), vortex all tubes, and spin briefly in a mini-spin centrifuge to collect the material at the bottom of each tube.
2. Assemble the reagents as shown in Table 2. Start by preparing the reaction mix for **X+2** reactions of Buffer A, Buffer B, and Buffer C (X = n samples + 1 negative control + 1 positive control)

Table 2: *EGFR* 6-color Crystal Digital PCR® Kit reaction mix assembly per reaction

Component Name	Initial Concentration	Final Concentration	Vol (µL) for 1 reaction
● Buffer A	10X	1X	0.60
● Buffer B	50X	1X	0.12
● Buffer C	8X	1X	0.75
<i>Buffer A, Buffer B, and Buffer C volume (µL)</i>			1.47
○ <i>diluted clinical sample or</i> ● <i>diluted positive control or</i> ○ <i>diluted negative control</i>			4.53
<i>Total reaction volume (µL)</i>			6.0

3. Vortex and spin the tube containing the reaction mix including Buffers A, B and C.
4. Split the content of this reaction mix into **X** tubes of 1.47 µL each.
5. Label tubes Neg Ctl, Pos Ctl and Samples (i.e. up to 45 DNA samples per full run). Stilla Technologies recommends following the order indicated below:
 - a) Preparation of the negative control reaction (**one reaction**):
 - Label one of the tubes containing 1.47 µL of reaction mix “Neg Ctl.”
 - Add 1.20 µL of negative control sample from the provided tube with the white cap.
 - Add 3.33 µL of the provided nuclease-free water.
 - b) Preparation of the sample reaction:
 - Add the desired sample volume to each of the individual sample tubes “Sample” (up to 45 per full run) containing 1.47 µL of reaction mix.
 - Add additionally nuclease free water to reach a final volume of 6 µL.
Note: Do not exceed a total DNA amount of 40 ng per reaction.
 - c) Preparation of the positive control reaction (**one reaction**):

It is recommended to end the preparation of the PCR mixes with the positive control reaction to avoid any risk of contamination of the clinical samples.

 - Label one of the tubes containing 1.47 µL of reaction mix “Pos Ctl.”
 - Add 1.20 µL of positive control sample from the provided tube with the purple cap.
 - Add additionally 3.33 µL of the provided nuclease-free water.
Note: adjust the number of reactions and respective volumes for the positive control as needed for a full run (recommended at least 2 two positive control chambers per full run).

6. Vortex each tube thoroughly to mix all reagents and briefly centrifuge to collect the content at the bottom of the tube.
7. Load 5 μ L of the reaction mix into 1 chamber of the Ruby Chip (refer to the Ruby Chip IFU for instructions to load a Ruby Chip). Recommended chip layout plan shown in Table 4.

Note: Stilla Technologies recommends loading first the negative control reaction, then loading the samples, and ending with the positive control. Do not pipet directly into the oil phase. Change tips after each pipetting.

Table 4: Example of recommended Ruby Chip layout plan

	Chip 1		Chip 2		Chip 3	
A	Sample 1	Sample 8	Sample 16	Sample 24	Sample 31	Sample 39
B	Sample 2	Sample 9	Sample 17	Sample 25	Sample 32	Sample 40
C	Sample 3	Sample 10	Sample 18	Sample 26	Sample 33	Sample 41
D	Sample 4	Sample 11	Sample 19	Sample 27	Sample 34	Sample 42
E	Sample 5	Sample 12	Sample 20	Sample 28	Sample 35	Sample 43
F	Sample 6	Sample 13	Sample 21	Sample 29	Sample 36	Sample 44
G	Sample 7	Sample 14	Sample 22	Sample 30	Sample 37	Sample 45
H	Neg Ctl	Sample 15	Sample 23	Pos Ctl.	Sample 38	Pos Ctl.

Step 2. Partition, Amplification, and Image Acquisition

Estimated duration of partitioning, amplification, image acquisition of the EGFR 6-color Crystal Digital PCR® Kit run on the Nio™+ for 3 Ruby Chips: 3 hours 15 min.

The following Nio™+ experiment for EGFR 6-color Crystal Digital PCR® Kit is available:

Table 5: Thermocycling and Reading programs parameters

1. Thermocycling	2. Reading
<p>Step 1 Partition for Ruby chip</p> <p>Step 2 Temperature 95°C for 180 seconds</p> <p>Step 3 Begin Loop for 20 iterations</p> <p>Step 3.1 Temperature 95°C for 15 seconds</p> <p>Step 3.2 Temperature 65°C for 30 seconds</p> <p>Step 4 Begin Loop for 40 iterations</p> <p>Step 4.1 Temperature 95°C for 15 seconds</p> <p>Step 4.2 Temperature 64°C for 30 seconds</p> <p>Step 5 Release for Ruby chip</p> <p>Rate of 1°C/sec for each step.</p>	

1. Open Nio™ Reader software.

2. Select “Open” in the menu “Experiments.” Under the box “Files”, click on “Add file” and browse the .nioprotocol and .nioassay for the *EGFR* 6-color Crystal Digital PCR® Kit.
 3. Drag and drop the .nioprotocol of the *EGFR* 6-color Crystal Digital PCR® Kit to the “Protocol” field underneath each chip plate layout according to desired experimental setup.
 4. Drag and drop the .nioassay of the *EGFR* 6-color Crystal Digital PCR® Kit to the chip plate layout according to desired experimental setup.
 5. Modify the sample name (NEG CTL, SAMPLE X, POS CTL) for each chamber.
 6. Select “Save As” to register the .nioexperiment to be used with the desired experimental setup with the *EGFR* 6-color Crystal Digital PCR® Kit. The saved .nioexperiment can be used as a template for future experiments with the same experimental setup.
 7. Select “Plan & Start” in the menu “RUNS”, browse and load the *EGFR* 6-color Crystal Digital PCR® Kit .nioexperiment file saved in previous step.
 8. Load the Chip Plate of the Ruby Chip consumables into the Nio™+.
 9. Select the “Start Run” button.
 10. Select “Status” in the menu “RUNS” to verify that the Chip Plate enters the “Thermocycling” step process. The Chip Plate will continue to be scanned once the thermocycling step is complete. Once a chamber is scanned, a quality flag appears in the chamber rectangle (refer to Nio™ Reader software User Manual for detailed instructions for checking image quality).
 11. At the end of run, copy the results file .niodata on a USB flash drive.
 12. To recover the chips, go to “Status” in the menu “RUNS” and select the “Nio Run” to unload. Click on “Unload Chip plate” on the right side of the user interface.
- Note:** *Ruby Chip consumables may be rescanned if necessary.*

Step 3. Data Analysis with Nio™ Analyzer

Please refer to Nio™ Analyzer software User Manual for instructions on general use.

1. Open the .niodata file generated by the Nio™ Reader software in the Nio™ Analyzer software.
2. Go to the menu “ANALYZE DATA > Plots & Populations > 2D dot plot”. If needed, double click on the graph to adjust the axis scales, and if not sufficient click on the blue gear below the 2D graph (indicated by the red arrow in Figure 4 below) to manually enter the desired value.

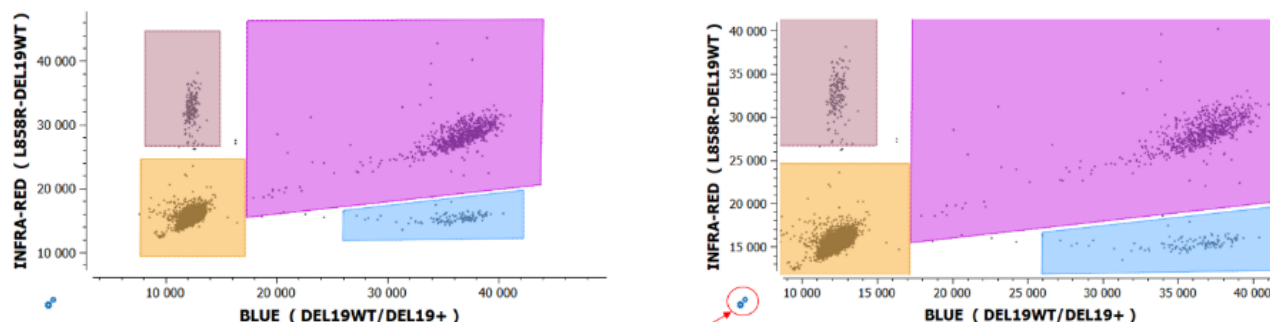


Figure 4: The Nio™ Analyzer software 2D dot plot for data visualization and analysis.

3. Check or manually adjust the position of the polygons for each target population listed in the “Populations” widget for the **positive control** of the run (refer to the example in Figure 5 below).

- To translate a polygon, left-click on an edge of the polygon and drag and drop it, then click on the “Apply” button to confirm.
- To move the vertex of a polygon, left-click on the vertex of the polygon and drag and drop it, then click on the “Apply” button to confirm.
- To cancel polygon adjustment, click on the “Cancel” button.

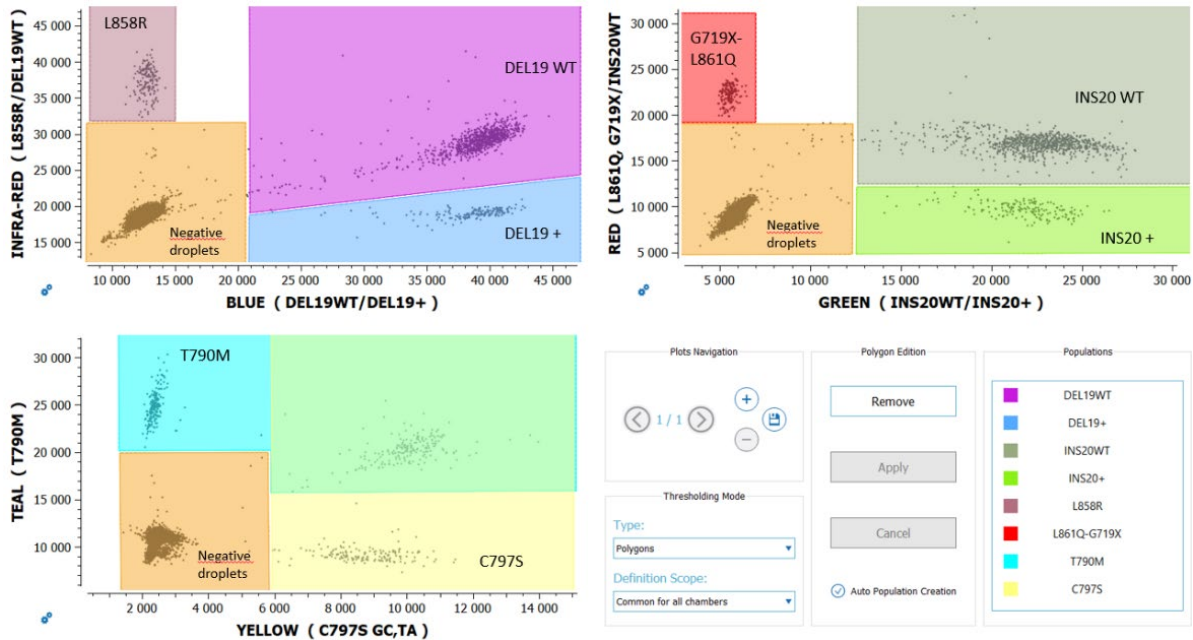


Figure 5: An example illustrating correct placement of the polygons on the positive control in the Nio™ Analyzer software.

4. In the thresholding mode, select “individual per chamber” for the definition scope.
5. Check or manually adjust the position of the polygons for each target population listed in the “Populations” widget for each sample. Polygons should now be correctly placed if the previous step is followed.
6. In case of **rare events** (<10 positive droplets) for a population of interest, confirm in the “Explore Crystals” submenu that each droplet classified as positive inside a polygon is a real positive droplet. If not, exclude it (refer to Nio™ Analyzer software User Manual for how to exclude droplets).
7. Go to “FILE > Save” to save the experiment in “.nioresult” format (under the same name as the experiment name).
8. Go to “VIEW RESULTS > Result Table” to access quantification results for each target population.
9. Optional: Go to the “EXPORT” menu and click on the “Browse” button to select the output folder, then click on the “Export” button to export the data in the “.xlsx” format (under the same name as the experiment name).

Note: The concentrations exported from Nio™ Analyzer are provided in copies per microliter for the given chamber. To calculate the amount of DNA in copies per microliter in the sample, multiply the exported concentration by the dilution factor.

$$\text{Dilution factor} = \text{total volume of 1 reaction (6 } \mu\text{L)} \div \text{sample volume (X } \mu\text{L)} = 6 \div X$$

*Sample volume (X) can be up to 4.53 μL .

Analytical Performances

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined following the Clinical and Laboratory Standards Institute (CLSI) EP17-A2 standard recommendations (*Protocols For Determination Of Limits Of Detection And Limits Of Quantitation; Approved Guideline*).

cfDNA samples

Table 6 indicates the LOB/LOD concentrations in one Ruby chamber for cfDNA samples in copier/ μ L. To compare them to the sample concentrations, multiply the LOB/LOD values by the sample dilution factor previously calculated, DNA sample volume divided by 6 μ L (total reaction volume).

Table 6: EGFR 6-color Crystal Digital PCR® Kit Limit of Blank (LOB) and Limit of Detection (LOD) in one Ruby chamber (copies/ μ L) for cfDNA samples

Target	LOB	LOD
DEL19+	0.32	1.27
L858R	0.28	0.97
INS20+	0.65	1.55
L861Q, G719X	0.62	1.63
T790M	0.57	1.64
C797S	0.29	1.29

FFPE samples

Table 7 indicates the LOB/LOD concentrations in one Ruby chamber for FFPE samples in mutant allele frequency (MAF, %).

Table 7: EGFR 6-color Crystal Digital PCR® Kit Limit of Blank (LOB) and Limit of Detection (LOD) in one Ruby chamber (MAF %) for FFPE samples

Target	LOB	LOD
DEL19+	1.5	2.0
L858R	1.5	2.0
INS20+	1.5	2.0
L861Q, G719X	1.5	2.0
T790M	1.5	2.0
C797S	1.5	2.0

Step 4. Interpretation of Sample Results

In the excel export file, in the “Results” tab, look at the total droplet number (“Nb Droplets”) and target concentration columns.

Table 8: Decision table

Target X concentration value	Interpretation
$C[X] \geq LOD$	Sample mutated for X
$LOB < C[X] < LOD$	Undetermined*
$C[X] \leq LOB$	Sample non-mutated for X

*For undetermined results, multiple chambers of the same sample can be pooled together to increase the total analyzable volume per sample. LOB/LOB of pooled Ruby chambers with cfDNA samples are available in Annex I. For otherwise undetermined result interpretation, Stilla Technologies advises to retest the sample in a new run and, if possible, increase the tested volume of the sample.

Mutant Allele Frequency (MAF) Calculation

$C[X]$ = target X concentration (copies/ μ L)

$C[DNA_{tot}]$ = Concentration of total DNA in the sample

$$= (C[Del19WT] + C[Del19+] + C[Ins20WT] + C[Ins20+]) / 2$$

$$X \text{ MAF} = C[X] * 100 / C[DNA_{tot}]$$

Run Data Validation

The run data with the *EGFR* 6-color Crystal Digital PCR® Kit is validated if:

1. Total number of droplets in each chamber is > 10,000 droplets
2. Negative control: Concentrations of all targets is \leq LOB
3. Positive control: Concentration of all targets must be within the range of concentrations defined in the CoC (Certificate of Compliance).
4. Measured amount of total DNA in a reaction (= $C[DNA_{tot}]$ x initial volume of sample) is less than 40 ng (~13.000 copies).

If measured amount of DNA_{tot} > 40ng, results are non-interpretable. Perform a new run with a decreased initial volume of sample. The concentrations measured in the first run should be used to adapt the volume of sample to be loaded in the reaction mix.

Quality Control

Each batch of *EGFR* 6-color Crystal Digital PCR® Kit is functionally tested on the Nio™+ and must perform within the established specifications. The Certificate of Compliance is available upon request from the Technical Support Team.

Precautions and Warnings

The reagents included in the *EGFR* 6-color Crystal Digital PCR® Kit are not classified as dangerous substances according to Regulation (EC) No. 1272/2008 [CLP].

Appropriate personal protection equipment for handling this product, including lab coat, disposable gloves, and safety goggles, is required. Wear additional personal protection equipment when needed. Wash hands before breaks and after work. Remove contaminated, saturated clothing.

In case of exposure:

- General information: when in doubt or if symptoms are observed, get medical advice.
- Following inhalation: no special measures are necessary. Provide fresh air.
- Following skin contact: wash with soap and water.
- Following eye contact: in case of eye irritation consult an ophthalmologist. Rinse immediately, carefully, and thoroughly with eyebath or water.
- Following ingestion: if swallowed: Rinse mouth. Do NOT induce vomiting.
- Self-protection of the first aider: no special measures are necessary.

For further information, please refer to the material safety data sheet available on the technical resources webpage.

Disposal Considerations

Waste can be considered as a biohazardous waste and must be disposed of according to applicable national or domestic legislation.

For recycling of cardboard packaging, please consult local or national regulations.

Annex I

In case of undetermined sample result interpretation, multiple chambers of the same sample can be pooled together to increase the total analyzable volume per sample. LOB/LOB of pooled Ruby chambers with cfDNA samples are available in Table 9.

When pooling in subsequent experiments, adjust the number of positive control and negative control chambers according to the number of sample chambers pooled.

Refer to Nio Analyzer section “Advanced functionalities” for details on how to pool and analyze pooled Ruby Chip chambers.

Table 9: EGFR 6-color Crystal Digital PCR® Kit Limit of Blank (LOB) and Limit of Detection (LOD) in one or pooled Ruby chambers (copies/μL) for cfDNA samples

Target:	DEL19+		INS20+		L858R		L861Q, G719X		T790M		C797S	
	LOB	LOD	LOB	LOD	LOB	LOD	LOB	LOD	LOB	LOD	LOB	LOD
1 Ruby Chamber	0.32	1.27	0.65	1.55	0.28	0.97	0.62	1.63	0.57	1.64	0.29	1.29
2 Ruby Chambers pooled	0.30	0.99	0.49	1.14	0.14	0.71	0.45	1.20	0.53	1.09	0.15	0.87
3 Ruby Chambers pooled	0.22	0.75	0.44	0.91	0.08	0.65	0.45	1.05	0.3	0.92	0.11	0.69
4 Ruby Chambers pooled	0.23	0.71	0.46	0.92	0.07	0.54	0.34	0.87	0.29	0.81	0.12	0.62

Technical Support Contact Information

Online Technical Support is available at: <https://www.stillatechnologies.com/technical-support/>

For technical questions or any issue regarding the *EGFR* 6-color Crystal Digital PCR® Kit, contact us:

Monday to Friday, 9:30 AM – 6:30 PM, Central European Time (CET).

Closed on French bank holidays.

Phone: (+33) 09 82 27 47 47

Email: support@stilla.fr

Patents: <https://www.stillatechnologies.com/patents/>

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