

**REF** 1141-011107-96

### DESCRIPTION

Primers and Probes set is specifically designed to be used with the QIAcuity 5 Plex dPCR system.

Primers and Probes for Mutants and wild-type targets detection are provided as two independent mixes to be used in a two-wells assay, with a combination of Green, Yellow, Red and Far Red probes. The lists of detectable targets for each tube are found in **Table 1a** and **1b**.

Additionally, the Primers and Probes set is enabled for the ID SOLUTIONS patented technology<sup>(1)</sup> in each test well. The Crimson channel is reserved for its detection.

**Table 1a:** List of detectable targets in **PRIMEX KRAS Ex2/3**

Exons	Targets	Base Exchanges	Detection Channel
Exon 2	Wild type	NA	GREEN / YELLOW <sup>(2)</sup>
	G12C	c.34_36 GGT>TGC	GREEN
		c.34 G>T	
		c.33_34T G>CT	
	G12D	c.35 G>A	
		c.35_36 GT>AC	
	G12V	c.35 G>T	
		c.35_36 GT>TC	
		c.34_35 GG>GT	
		c.35_36 GT>TG	
	G12R	c.34 G>C	
		c.34_36 GGT>AGA	
	G12S	c.34 G>A	
		c.34_35 GG>TC	
	G12A	c.35 G>C	
		c.35_36 GT>CA	
	G12F	c.34_35 GG>TT	
	G13C	c.37 G>T	
		c.36_37T G>AT	
	G13D	c.38 G>A	
c.38_39 GC>AT			
G13R	c.37 G>C		
	c.36_37T G>AC		
G13S	c.37 G>A		
Exon 3	Wild type	NA	FAR RED / RED <sup>(2)</sup>
	Q61R	c.182 A>G	FAR RED
	Q61L	c.182 A>T	

Exon 3	Q61E	c.181 C>G	FAR RED
	Q61H	c.183 A>C	
		c.183 A>T	
	Q61K	c.181 C>A	
		c.180_181T C>AA	
Q61P	c.182 A>C		
-	ICE <sup>(1)</sup>	NA	CRIMSON

<sup>(2)</sup> The channel highlighted in bold is the channel used for the analysis of the target population using the lasso partitions assignment tool when two fluorophores are involved in the detection.

For samples with template inputs > 20ng or mutation frequencies > 20%, please refer to the Results Analysis section.

**Table 1b:** List of detectable targets in **PRIMEX KRAS Ex4**

Exons	Targets	Base Exchanges	Detection Channel
Exon 3	Wild type	NA	RED
Exon 4	A146T	c.436 G>A	GREEN
	A146P	c.436 G>C	
	A146V	c.437 C>T	
	K117N	c.351 A>C	YELLOW
c.351 A>T			
-	ICE <sup>(1)</sup>	NA	CRIMSON

### STORAGE & STABILITY

Reagents are stable for the shelf life claimed on the product labeling when stored between -26°C and -16°C protected from light.

### CONTENTS

Each package contains:

- 4 tubes of primers and probes **PRIMEX KRAS Ex2/3** (Ref. SF3080-1141-000046-1-24; White cap; 120µL each), sufficient for up to 24 reactions each,
- 4 tubes of primers and probes **PRIMEX KRAS Ex4** (Ref. SF3080-1141-000007-2-24; Blue cap; 120µL each), sufficient for up to 24 reactions each,
- 2 tubes of target process control **TPC KRAS 3X** (Ref. SF3030-1141-000007-1-10; Red cap; 350µL each), sufficient for 10 reactions each.

### REAGENT & EQUIPMENT

To use Primers and Probes set, the following is required but not included:

- QIAcuity 5 Plex dPCR system (with QIAcuity

Software Suite 3.1)

- QIAcuity High Multiplex Probe PCR Kit (Cat no. 250133, 250134)

- QIAcuity Nanoplate 26K 8-well (Cat no. 250031) or QIAcuity Nanoplate 26K 24-well (Cat no. 250001)

- Optional: companion product (ID-ICE; ref. 1171-000000-96)

## REACTION SETUP

**Thaw** all components and let them set to room temperature. Mix thoroughly by vortexing each tube to ensure homogeneity. Centrifuge briefly to collect content at the bottom of each tube and store protected from light.

- 1. Reconstitute** the Amplification Reaction Mixture (ARM) by adding **250 µL** of QIAcuity High Multiplex Probe PCR Kit mastermix in one vial of each **PRIMEX (Ex2/3 and Ex4)**. Mix thoroughly by vortexing and centrifuge briefly to ensure that all components are at the bottom of each tube.
- 2. Prepare** samples at the desired concentration before setting up the reaction mixture according to **Table 2**. It is recommended to include a no-template control (NTC) in each run, prepared with nuclease-free water instead of DNA, to monitor for potential contamination introduced during sample preparation, pipetting, or amplification. The NTC should be processed and analyzed under the same conditions as the test samples. **Process** the **TPC** as a sample. It should be used to validate the test. Expected results are shown in **Figure 1** and **Figure 2**.
- 3. Setup** the dPCR reactions as described in **Table 2** for each **PRIMEX (Ex2/3 and Ex4)** and make sure that the ARM and the sample are well homogenized.
- 4. Transfer** the reaction mix from the reaction tubes to the QIAcuity Nanoplate 26K wells. Using one well for each **PRIMEX (Ex2/3 and Ex4)**.
- 5. Follow** instructions for thermal cycling described in **Table 3**.

## SIGNAL THRESHOLDING

Before analyzing results, ensure that the preset auto-thresholds are reviewed and, if necessary, depending on the assay, either manually adjusted or refined using the lasso function to accurately assign single positive and negative partitions.

These adjustments should be based on the signal populations shown in the 2D scatterplots in the Process Control Validation section below.

For detailed instructions on using the lasso function, please refer to the QIAcuity User Handbook.

**Table 2:** Setup of dPCR reaction mix

Component	Volume per reaction	Final concentration
ARM	15 µL	1X
Sample	25 µL	Up to 100 ng
Total Volume	40 µL	-

**Table 3:** Thermal cycling profile

Steps	Temperature	Time	Steps
Primex activation	50	2 min	1
Polymerase activation	95	2 min	1
DNA denaturation	95	15 sec	50
Annealing/Extension	60	30 sec	

## DATA ACQUISITION

Follow instructions for data acquisition and analysis based on QIAcuity user manual.

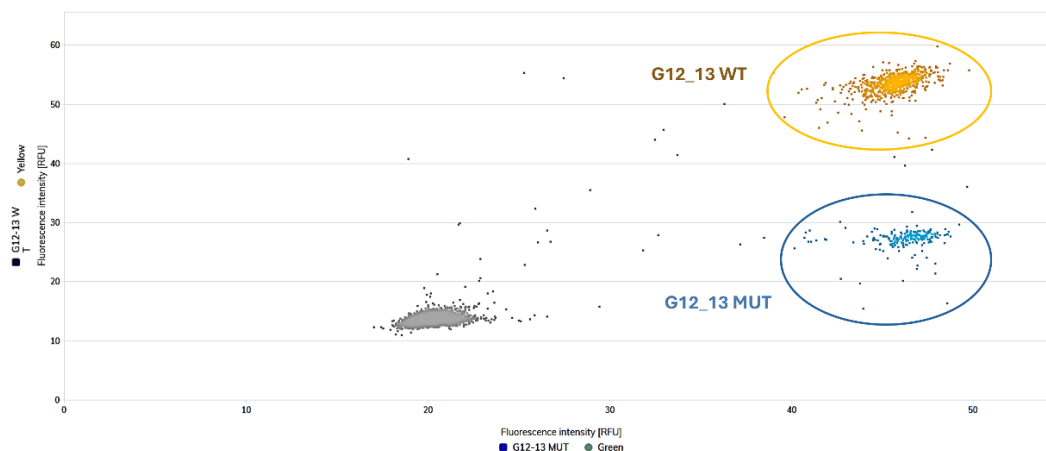
Set integration time and gain as described in **Table 4**.

**Table 4:** Imaging settings

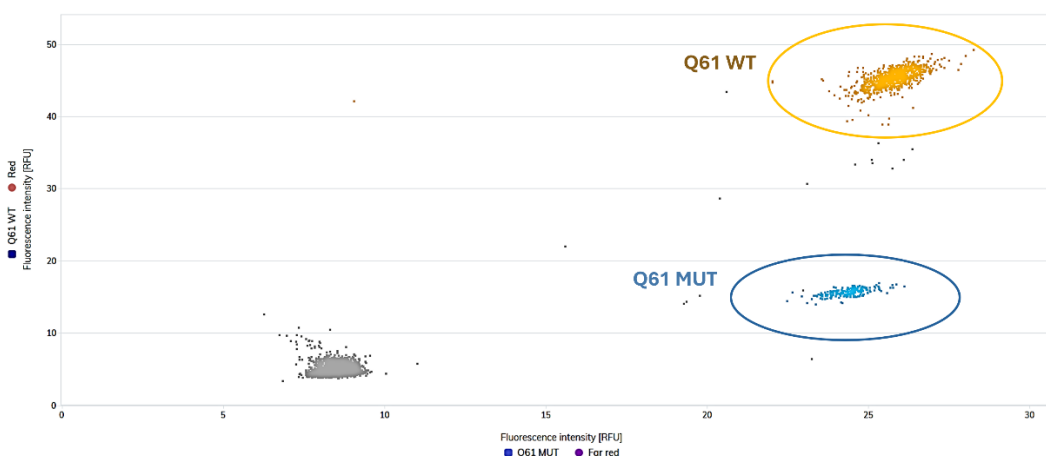
Channel	Integration (ms)	Gain
Green	300	6
Yellow	250	4
Orange	---	-
Red	150	3
Far Red	600	8
Crimson	300	4

## PROCESS CONTROL VALIDATION

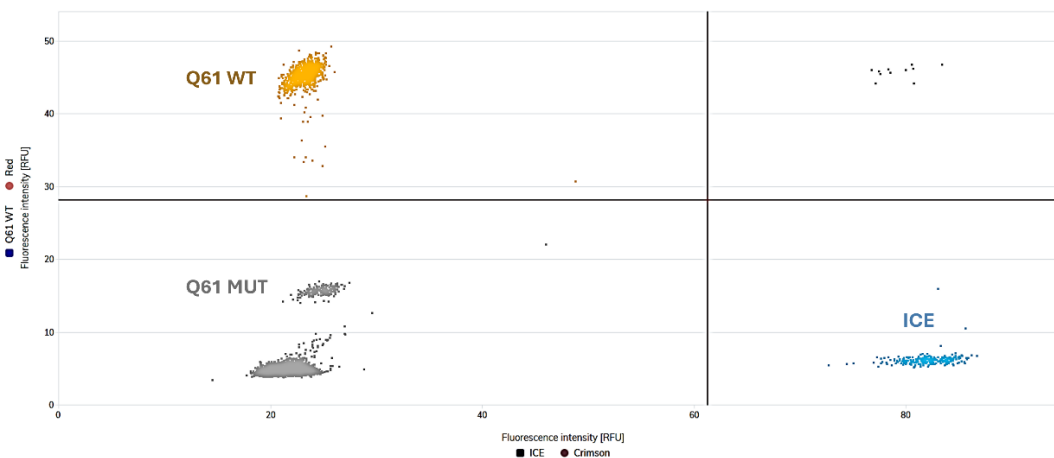
The profile of the TPC should be used to validate the test. When tested on gDNA extracted from tissue with **PRIMEX KRAS Ex2/3**, the TPC is best analyzed in the configuration shown in **Figure 1a** and **Figure 1b**. When tested on cfDNA extracted from liquid specimen with **PRIMEX KRAS Ex2/3**, including the use of ID-ICE, the TPC is best analyzed in the configuration shown in **Figure 1a**, **Figure 1b** and **Figure 1c**.



**Figure 1a:** TPC profile in Yellow (y-axis) and Green (x-axis) in association with PRIMEX KRAS Ex2/3. Populations are identified using the lasso partitions assignment tool in accordance with the detection channel assign in table 1.

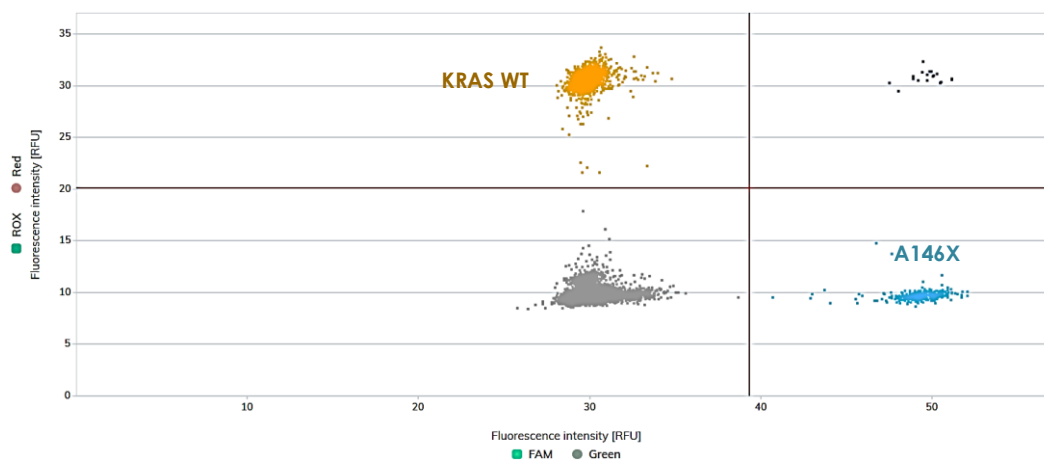


**Figure 1b:** TPC profile in Red (y-axis) and Far Red (x-axis) in association with PRIMEX KRAS Ex2/3. Populations are identified using the lasso partitions assignment tool in accordance with the detection channel assign in table 1.

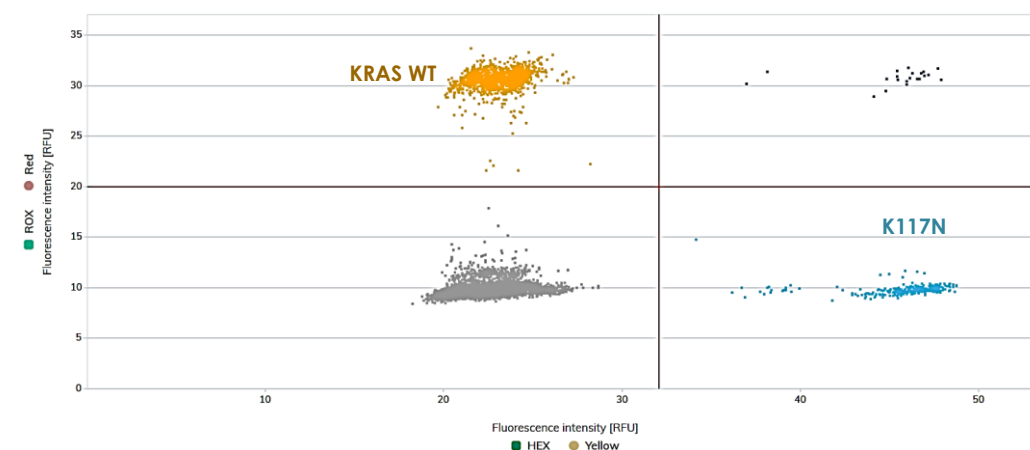


**Figure 1c:** TPC profile in Red (y-axis) and Crimson (x-axis) in association with PRIMEX KRAS Ex2/3. Populations are identified using the thresholding method in accordance with the detection channel assigned in table 1.

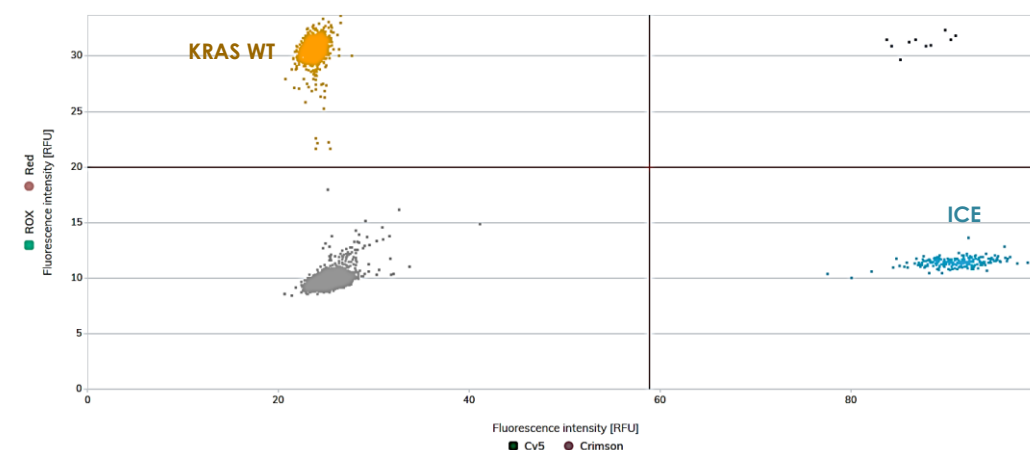
The profile of the TPC should be used to validate the test. When tested on gDNA extracted from tissue with **PRIMEX KRAS Ex4**, the TPC is best analyzed in the configuration shown in **Figure 2a** and **Figure 2b**. When tested on cfDNA extracted from liquid specimen with **PRIMEX KRAS Ex4**, including the use of ID-ICE, the TPC is best analyzed in the configuration shown in **Figure 2a**, **Figure 2b** and **Figure 2c**.



**Figure 2a:** TPC profile in Red (y-axis) and Green (x-axis) in association with PRIMEX KRAS Ex4. Populations are identified using the thresholding method in accordance with the detection channel assigned in table 1.



**Figure 2b:** TPC profile in Red (y-axis) and Yellow (x-axis) in association with PRIMEX KRAS Ex4. Populations are identified using the thresholding method in accordance with the detection channel assigned in table 1.



**Figure 2c:** TPC profile in Red (y-axis) and Crimson (x-axis) in association with PRIMEX KRAS Ex4. Populations are identified using the thresholding method in accordance with the detection channel assigned in table 1.

## RESULTS ANALYSIS

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After reviewing and confirming the thresholds, results can be analyzed for presence/absence of each target and calculation of fractional abundance using one of the following methods:

1. **Copies/ $\mu$ l Concentrations** – Accessible directly in the List view within the QIAcuity Software Suite. This method is ideal for low to medium input samples (up to 20 ng, approximately 6,000 total copies per reaction) and mutation frequencies up to 20%.
2. **Multiple Occupancy Export Table** – Recommended for all assays with at least one population detected with 2 detection channels. This approach is best suited for accurate quantification of fractional abundances in high input samples (> 20 ng) and mutation frequencies exceeding 20%.

The **Multiple Occupancy Export Table** provides corrected concentrations by accounting for the random colocalization of multiple templates within the same partition. Signal combinations are displayed using a presence (+) or absence (-) notation. For example, with two detection channels — green and yellow — you may observe the following combinations: ++ (both signals), +- (green only), -+ (yellow only), and -- (no signal). For accurate calculations, double-positive partitions must be assigned as double positive in the 2D scatterplot using the lasso assignment tool. Refer to Table 1 to assign each target to its corresponding detection channel(s).

Fractional abundance may be estimated according to the following formula:

$$G12_{13} \text{ MUT \%AF} = \frac{[G12_{13} \text{ MUT}]}{[G12_{13} \text{ WT}] + [G12_{13} \text{ MUT}]} \times 100$$

$$Q61 \text{ MUT \%AF} = \frac{[Q61 \text{ MUT}]}{[Q61 \text{ WT}] + [Q61 \text{ MUT}]} \times 100$$

$$A146X \text{ \%AF} = \frac{[A146X]}{[KRAS \text{ WT}]} \times 100$$

$$K117N \text{ \%AF} = \frac{[K117N]}{[KRAS \text{ WT}]} \times 100$$

## PERFORMANCE & VALIDATION

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The performance of the test should be validated prior to use.

The product has been validated on nucleic acids extracted using the EZ2 Connect extraction system, with both cfDNA and FFPE extraction kits.

## OPTIONAL STEP

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A companion reagent (ICE<sup>(1)</sup>) may be implemented to specifically validate the extraction procedure of each test sample. The procedure requires a product that can be ordered separately (ID-ICE, ref.: 1171-000000-96). This reagent is a non-human and non-GMO fragment. It should be spiked in samples prior to extraction and is co-amplified together with the other targets in the same well. The Crimson channel is used for its detection.

Please contact [info@id-solutions.fr](mailto:info@id-solutions.fr) for more information.

<sup>(1)</sup> ID SOLUTIONS patented technology, Pat. n°FR3061720 / WO2018/127674.


**ID SOLUTIONS**

585 rue de la Croix Verte – 34090 Montpellier - FRANCE  
 +33 (0)4 67 79 72 60 | [info@id-solutions.fr](mailto:info@id-solutions.fr) | [www.id-solutions.fr](http://www.id-solutions.fr)

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**SYMBOLS**

	Contains sufficient for < n > tests		Manufacturer
	Batch code		Keep away from the sunlight
	Catalog number		Temperature limit
	Use-by date		Caution
	Positive control		
	Consult instructions for use or consult electronic instructions for use		

**REVISION HISTORY**

VERSION	CHANGES
MAN_1141-011107_EN_V1.0	Creation
MAN_1141-011107_EN_V2.0	Addition of the mention of the software QIAcuity Software Suite 3.1 compatible with QIAcuity High Multiplex Probe PCR Kit Modification PRIMEX KRAS Ex2/3 channel for exon 3
MAN_1141-011107_EN_V2.1	Update using template MAN_1141-01_EN_V1.3
MAN_1141-011107_EN_V2.2	Color correction of PRIMEX Ex2/3 cap
MAN_1141-011107_EN_V2.3	Precision concerning the two different PRIMEX used in a two-wells assay