IDNAPTEX KRAS G12C

Primers and Probes set for KRAS Mutation detection

REF 1141-011108-96

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 mixed and provided in a single tube, with a combination of Green and Yellow probes. The list of detectable targets is found in **Table 1**.

Additionally, the Primers and Probes set is enabled for the ID SOLUTIONS patented technology[1] in each test well. The Crimson channel is reserved for its detection.

Table 1: List of detectable targets

Exons	Targets	Base Exchanges	Detection Channel
Exon 3	Wild type	NA	YELLOW
Exon 2	G12C	c.34_36GGT>TGC	GREEN
		c.34G>T	
		c.33_34TG>CT	
-	ICE[1]	NA	CRIMSON

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

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 G12C** (Ref. SF3080-1141-000008-1-24; Blue cap; 120µL each), sufficient for up to 24 reactions each,
- 2 tubes of target process control **TPC KRAS G12C** (Ref. SF3030-1141-000008-1-10; 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 Probe PCR Kit (Cat no. 250101, 250102, 250103)
- 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 Probe PCR kit mastermix in one vial of **PRIMEX**. 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.
3. **Process** the **TPC** as a sample. It should be used to validate the test. Expected results are shown in **Figure 1**.
4. **Setup** the dPCR reaction as described in **Table 2** and make sure that the ARM and the sample are well homogenized.
5. **Transfer** the reaction mix from the reaction tubes to the QIAcuity Nanoplate 26K wells.
6. **Follow** instructions for thermal cycling described in **Table 3.**

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	500	6
Yellow	500	6
Orange	---	-
Red	---	-
Far Red	---	-
Crimson	400	8

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.

PROCESS CONTROL VALIDATION

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

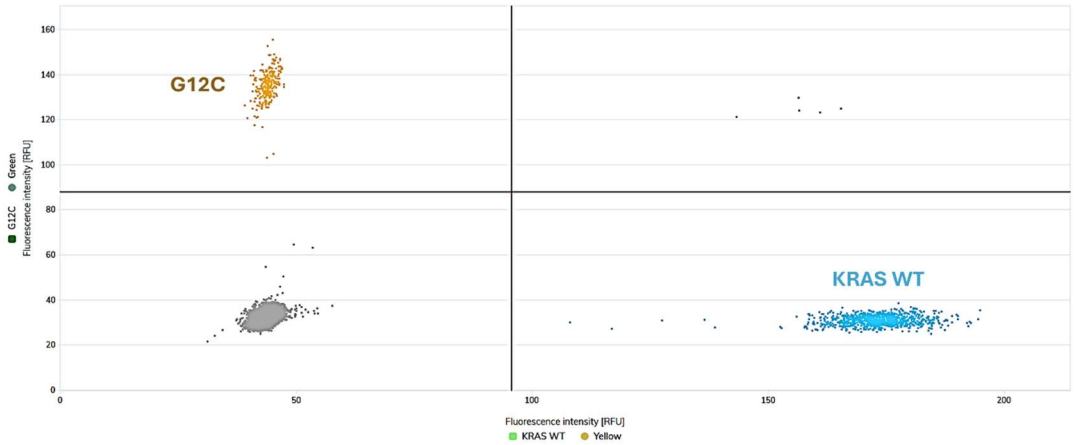

Figure 1a: TPC profile in Green (y-axis) and Yellow (x-axis). Populations are identified using the thresholding method in accordance with the detection channel assigned in table 1.

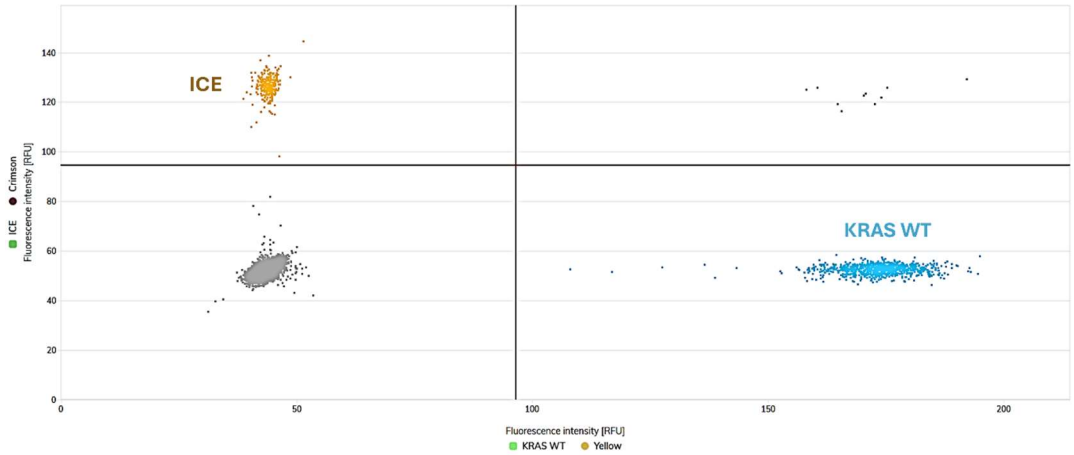

Figure 1b: TPC profile in Crimson (y-axis) and Yellow (x-axis) Populations are identified using the thresholding method in accordance with the detection channel assigned in table 1.

RESULTS ANALYSIS

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/µl 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:

$$G12C\ \%AF = \frac{[G12C]}{[Wild\ Type]} \times\ 100$$

PERFORMANCE & VALIDATION

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

A companion reagent (ICE[1]) 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 for more information.

[1] ID SOLUTIONS patented technology, Pat. n°FR3061720 / WO2018/127674.

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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_1141-011108_EN_V1.0	Creation
MAN_1141-011108_EN_V1.1	Update from template MAN_1141-01_EN_V1.2
MAN_1141-011108_EN_V1.2	Update from template MAN_1141-01_EN_V1.3