



miRpredX 31-3p

For in vitro diagnostic only

Instructions for use

Version 8



Please read carefully before using the product.

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1- miRpredX 31-3p kit

1.1- Indications for use

The miRpredX 31-3p CE-IVD in vitro diagnostic kit is designed to quantify the expression of the microRNA miR-31-3p in FFPE samples from primary tumors obtained from patients with metastatic colorectal cancer (mCRC). The miRpredX 31-3p test predicts the potential clinical benefits associated with first-line anti-EGFR (epidermal growth factor receptor) therapy compared to anti-VEGF (vascular endothelial growth factor) therapy or when second or further lines of treatment with anti-EGFR therapy is beneficial versus chemotherapy alone for patients with RAS wild-type (WT) mCRC.¹⁻³

Results from the miRpredX 31-3p test are intended to support therapeutic decisions for RAS WT mCRC patients.

The miRpredX 31-3p kit should only be utilized by trained laboratory professionals who have experience in handling RNA samples and RNA extracted from FFPE (formalin fixed paraffin embedded) tissues.

1.2- Instructions for use

The miRpredX 31-3p test kit should be used in conjunction with or after testing for the determination of a patient's tumoral RAS mutation status.

The miRpredX 31-3p kit is not intended to be a diagnostic test for colorectal cancer or mCRC.

miRpredX 31-3p should be utilized as a therapeutic decision aid for patients with metastatic colorectal cancer only. It is not intended for use in other types of cancer.

2- Concept design for miR-31-3p biomarker quantification test

2.1- Metastatic Colorectal Cancer (mCRC)

Colorectal cancer is third most common cancer in men and second most common in women on a worldwide basis.⁴ An estimated 25% of patients with colorectal cancer will present with metastatic disease with up to an additional 50% of patients developing mCRC following initial diagnosis.⁵

2.2- KRAS and NRAS

Approximately 50% of patients with metastatic colorectal cancer have RAS (KRAS or NRAS) mutated tumors.⁶ Since results from published studies and current guidelines associated with the management of patients with mCRC indicate that patients with RAS mutations have a poor response to anti-EGFR therapy, these agents are not recommended in this patient population.⁶⁻⁹ Anti-EGFR therapy does remain an option for RAS WT mCRC patients, although, not all of these patients respond to this therapy.

2.3- miR-31-3p predicts response to anti-EGFR therapy in RAS WT mCRC patients

Clinical studies have demonstrated that miR-31-3p expression levels in primary tumors of wild type RAS patients predict response to anti-EGFR treatment.¹⁻³ miR-31-3p level of expression measurement in addition to the determination of RAS mutational status in mCRC patients can enable clinicians to identify treatment options which are potentially associated with improved therapy outcomes.

A recent study has validated a cut-off value of 1.36 which differentiates low miR-31-3p from high miR-31-3p expressors.² RAS WT mCRC patients whose tumors have a low expression of miR-31-3p ($RQ < 1.36$) have an improved response to anti-EGFR therapy when used in first-line when compared to anti-VEGF therapy. Low miR-31-3p expressors also have a better response to anti-EGFR therapy versus chemotherapy alone when used for second or further lines of treatment. RAS WT mCRC patients whose tumors have a high expression of miR-31-3p ($RQ \geq 1.36$) have a similar response to first-line anti-EGFR and anti-VEGF therapy. High miR-31-3p expressors also have a better response to chemotherapy alone versus anti-EGFR therapy when used for second or further lines of treatment.

2.4- Description

The miR-31-3p kit is designed to quantify the expression of the microRNA miR-31-3p in FFPE samples from tumors. The test measures the expression level of miR-31-3p and compares this with the expression of a housekeeping microRNA (calibrator). Measurement of both miR-31-3p and calibrator expression is performed by retro-transcription followed by real time PCR (RT-qPCR) quantification.^{10,11} The microRNA calibrator is a housekeeping miRNA which is not affected by the pathology and is utilized to normalize the assay and reduce sample preparation associated bias (extraction, total quantity of total RNA input in reverse transcription reaction).

The miRpredX 31-3p kit uses RT-qPCR to measure miR-31-3p and the microRNA calibrator using two FAM-labelled specific probes.

2.4.1- General considerations

Real-time PCR is based on detection and quantification of a fluorescent “reporter”. The increase of fluorescence signal is only detected if an amplified sequence is recognized by complementary probe. Using this method insures the fluorescence signal is directly related to target amplification during PCR and that non-specific amplification is not detected.

Amplification is visually represented by a sigmoid curve based on 3 steps:

- 1- The background noise step - when amplification is not sufficient to generate a detectable signal with the technique utilized.*

- 2- The exponential amplification step - when PCR product quantity produced at each step is directly related to starting material copy number. The starting amplification step begins when the signal becomes detectable above the background noise and corresponds to number of cycles, identified as Ct (Cycle threshold).
- 3- The plateau step – this step follows exponential amplification and is reached when there is a lack of reagents remaining.

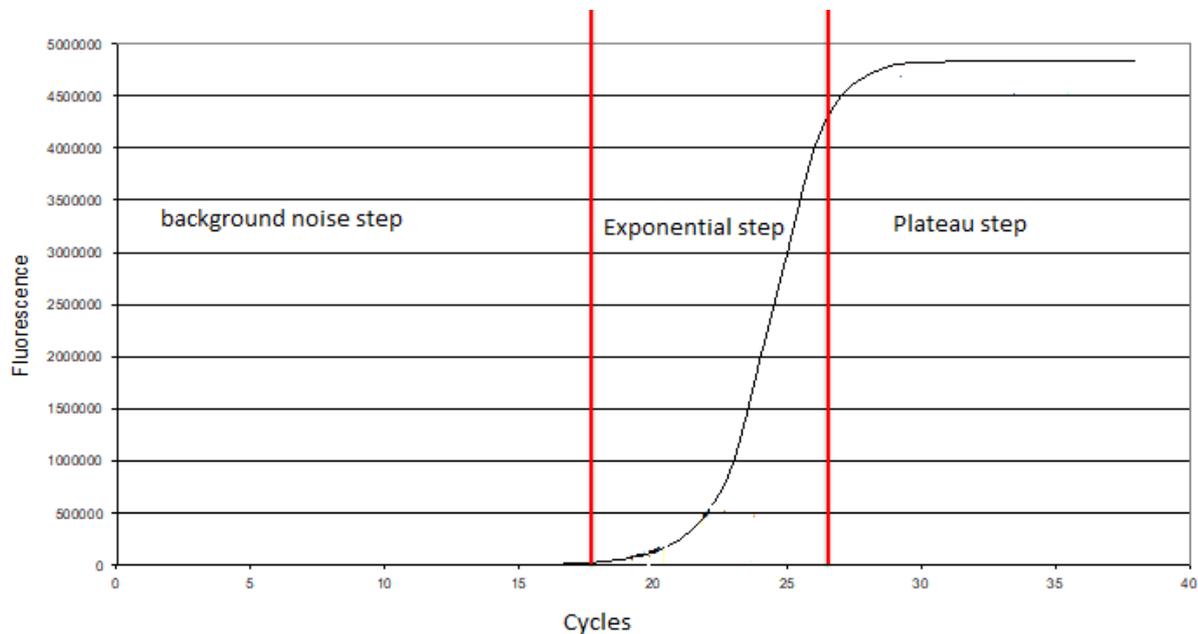


Figure 1: Typical output of real time-PCR showing an increase of fluorescent signal (y-axis in logarithmic scale) for each PCR cycle (x-axis). Units of scale for the y-axis (ΔRn) is the fluorescence signal normalized against a reference passive dye present in reaction master mix.

2.4.2- miRpredX 31-3p test description

Both miR-31-3p and the microRNA calibrator are measured using two FAM-labelled specific probes (abs.: $\lambda = 494 \text{ nm}$). Results are calculated utilizing a standard dilution curve based on four known concentrations of both miR-31-3p and the microRNA calibrator.

Two standards curves, one specific for miR-31-3p and one for the microRNA calibrator, are used to calculate efficiency and the linearity of qPCR measurement and to quantify relative concentrations of the microRNAs in each sample. Specific ranges for the correlation coefficient (r^2) and the standard curve slope are used as criteria for test acceptance for the qPCR assay. A value of between 0.95 and 1.00 is the acceptance criteria for r^2 with a value of $r^2 > 0.98$ being preferred. The standard slope target is -3.32 (between -3.92 and -2.92, inclusive).

A negative control should always be performed.

Ct represents the number of cycles necessary to achieve detectable fluorescence. The higher the *Ct*, the less the number of copies of the target microRNA are present in sample. *Ct* values are negatively correlated with number of molecules measured in sample.

The difference between miR-31-3p *Ct* and the miR calibrator *Ct* for each sample is compared to the difference between miR-31-3p *Ct* and the miR-calibrator *Ct* for a reference standard dilution. This method, described as $\Delta\Delta Ct$, enables the calculation of RQ with RQ defined as the level of microRNA expression.¹² The equation for calculating RQ is as follows: $RQ = 2^{-\Delta\Delta Ct}$.

Example of Ct comparison

Step 1: Standard calculation (S2 Standard)

$$Ct_{miRNA\ target} - Ct_{control} = \Delta Ct_{standard}$$

(This value is the same for all standard curve dilutions)

Step 2: Normalization of sample against control

$$Ct_{miRNA\ target} - Ct_{control} = \Delta Ct_{sample}$$

Step 3: Normalization against standard

$$\Delta Ct_{sample} - \Delta Ct_{standard} - K^* = \Delta\Delta Ct$$

Step 4: RQ Calculation

$$RQ = 2^{-\Delta\Delta Ct}$$

* The analytical factor for the standard: for kit batch number #IG170801 to #IG181204, K value is 1.43.

Example calculation 1: Low miR-31-3p expression

Reference Standard:

miR-calibrator: average Ct = 24.03

$$\Delta Ct_{\text{standard}} = 26.9 - 24.03 = 2.87$$

miR-31-3p: average Ct = 26.90

Results from Patient Tumor Sample:

miR-calibrator Ct = 28.01

$$\Delta Ct_{\text{patient}} = 32.59 - 28.01 = 4.57$$

miR-31-3p Ct = 32.59

$$\Rightarrow \Delta \Delta Ct = (4.57 - 2.87) - 1.43 = 0.27$$

$$RQ = 2^{-\Delta \Delta Ct} = 2^{-0.27} = 0.83$$

The results in the above example indicate the number of miR-31-3p copies is 0.83 times lower in the patient's tumor sample compared to the control. Since the cut-off for differentiating low versus high expressor for miR-31-3p is 1.36, this patient would be considered a low expressor of miR-31-3p.

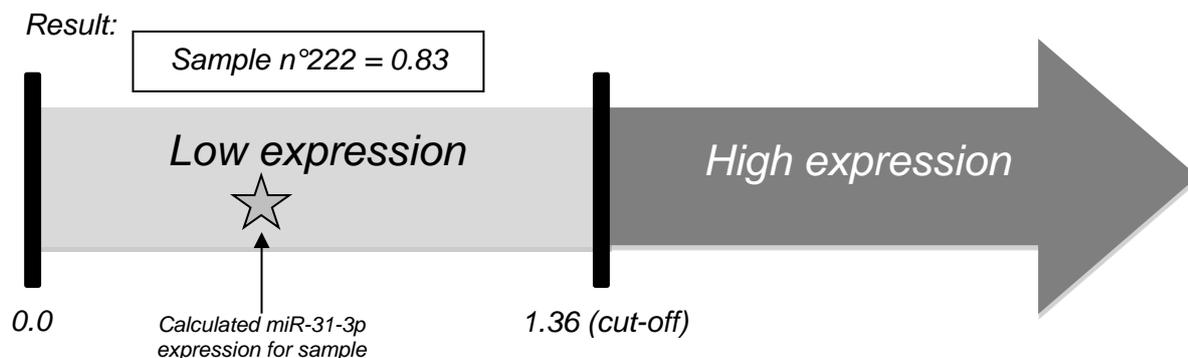


Figure 2: Graphical representation of the results from Example calculation 1.

Example calculation 2: High miR-31-3p expression

Reference Standard:

miR-calibrator: average Ct = 24.03

$$\Delta Ct_{\text{standard}} = 26.9 - 24.03 = 2.87$$

miR-31-3p: average Ct = 26.9

Results from Patient Tumor Sample:

miR-calibrator Ct = 23.50

$$\Delta Ct_{\text{patient}} = 25.83 - 23.50 = 2.33$$

miR-31-3p Ct = 23.83

$$\Rightarrow \Delta \Delta Ct = (2.33 - 2.87) - 1.43 = -1.97$$

$$RQ = 2^{-\Delta \Delta Ct} = 2^{1.97} = 3.97$$

The results in the above example indicate the number of miR-31-3p copies is 3.97 times higher in the patient's tumor sample compared to the control. Since the cut-off for differentiating low versus high expression for miR-31-3p is 1.36, this patient would be considered a high expresser of miR-31-3p.

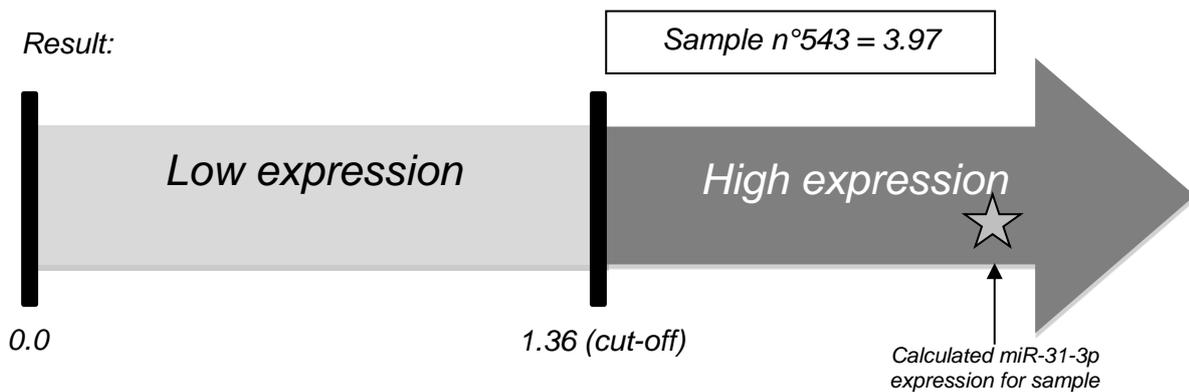


Figure 3: Graphical representation of the results from Example calculation 2.

2.5- Workflow for patient samples

The miR-31-3p expression test is intended to be utilized in conjunction with KRAS and NRAS mutational testing. The same set of slides from the FFPE tumor block should be utilized for both RAS mutation and miR-31-3p expression testing.

2.5.1- RAS Mutation Testing Workflow with miRpredX 31-3p Expression Testing

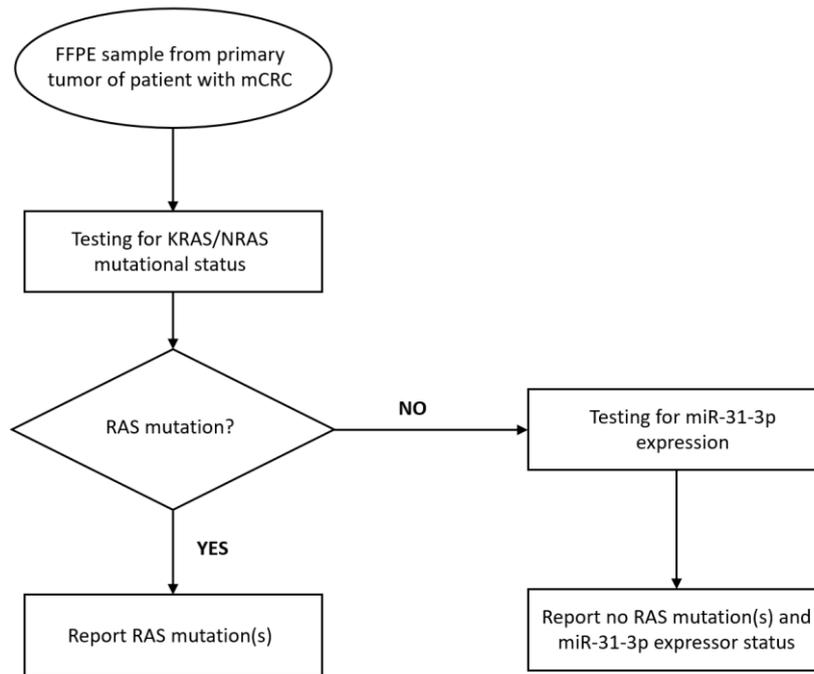


Figure 4: Representation of possible integrated workflow for KRAS / NRAS mutational testing in conjunction with miR-31-3p expression testing with the miRpredX 31-3p kit.

2.5.2- miR-31-3p expression Testing - Procedure overview

Prior to miR-31-3- expression analysis, tumor tissue from FFPE slides is removed by scratching and then processed via RNA extraction. Small and microRNAs will also be extracted during this process.

Pre-analytical step	RNA extraction (including small and microRNAs) from FFPE tumor tissue. <i>Important note: reagents for this step are not included in miRpredX kit.</i>	
Analytical steps 	Step	Approximate time required to complete step
	Plate layout set-up	15 minutes
	Run reverse transcription	75 minutes
	qPCR plate setup	30 minutes
	Run PCR	90 minutes
Data analysis	20 minutes	

Figure 5: Workflow for miRpredX 31-3p test.

3- miRpredX 31-3p Kit Contents

Reagent	Description	Form	Cap color	Cap code	Content	Storage
Standard Curve components						
<i>Standard 1</i>	<i>Standard (100ng tRNA)</i>	<i>Liquid</i>	<i>White</i>	<i>S1</i>	<i>10 µL</i>	<i>-15°C to -25°C</i>
<i>Standard 2 (reference)</i>	<i>Standard (50ng tRNA)</i>	<i>Liquid</i>	<i>White</i>	<i>S2</i>	<i>10 µL</i>	<i>-15°C to -25°C</i>
<i>Standard 3</i>	<i>Standard (5ng tRNA)</i>	<i>Liquid</i>	<i>White</i>	<i>S3</i>	<i>10 µL</i>	<i>-15°C to -25°C</i>
<i>Standard 4</i>	<i>Standard (0.5ng tRNA)</i>	<i>Liquid</i>	<i>White</i>	<i>S4</i>	<i>10 µL</i>	<i>-15°C to -25°C</i>
RT Reagents						
<i>NTP Premix A</i>	<i>Buffer and dNTP</i>	<i>Liquid</i>	<i>Blue</i>	<i>RT1</i>	<i>60 µL</i>	<i>-15°C to -25°C</i>
<i>RT Premix B</i>	<i>Reverse transcriptase and RNase inhibitor</i>	<i>Liquid</i>	<i>Blue</i>	<i>RT2</i>	<i>40 µL</i>	<i>-15°C to -25°C</i>
<i>miR-31-3p primers</i>	<i>Primers</i>	<i>Liquid</i>	<i>Blue</i>	<i>PI</i>	<i>50 µL</i>	<i>-15°C to -25°C</i>
<i>miR-calibrator primers</i>	<i>Primers</i>	<i>Liquid</i>	<i>Blue</i>	<i>PN</i>	<i>50 µL</i>	<i>-15°C to -25°C</i>
PCR Reagents						
<i>qPCR Master Mix</i>	<i>Master Mix qPCR, no UNG</i>	<i>Liquid</i>	<i>Red</i>	<i>qPCR MM</i>	<i>980 µL</i>	+2°C to +8°C
<i>miR-31-3p probe</i>	<i>Probe</i>	<i>Liquid</i>	<i>Red</i>	<i>AI</i>	<i>100 µL</i>	<i>-15°C to -25°C</i>
<i>miR-calibrator probe</i>	<i>Probe</i>	<i>Liquid</i>	<i>Red</i>	<i>AN</i>	<i>100 µL</i>	<i>-15°C to -25°C</i>
<i>Water, nuclease-free</i>	<i>Water for molecular biology (sterile, nuclease free)</i>	<i>Liquid</i>	<i>Red</i>	<i>H2O</i>	<i>750 µL</i>	<i>-15°C to -25°C</i>

Table 1: kit contents

4- Number of samples analyzed per miRpreX 31-3p kit

The miRpredX 31-3p kit is designed to perform the analysis of 12 samples. 4 vials containing the reagents need to perform a standard curve are supplied with the kit. It is important to perform a standard curve for each batch of samples. The miR-31-3p kit can be used up to 3 times (3 freeze/thaw cycles are possible) with 3 sets of 4 patient samples run and 1 standard curve per batch. A single RT reaction should be performed for each sample. The qPCR step should be performed in duplicate for each sample.

5- Required equipment and additional reagents

Necessary equipment and reagents for determining miR-31-3p expression which are not included in the miRpredX kit are as follows:

- RNA extraction kit that also extracts small and microRNA (< 200 nt) (see. § 8.3)
- 0.2 mL microtubes RNase free
- 1.5 mL microtubes
- 0.2 mL strips - optional
- 96 (or 384) well plate and seal intended for use with thermocycler
- 96 well plate and PCR seal intended for use with real-time PCR equipment
- 1-10 µL, 2-20 µL, 10-100 µL, 20-200 µL and 100-1000 µL pipettes (2 sets are recommended; 1 set for RNA handling [Reverse Transcription] and 1 set for DNA [qPCR])
- 10 µL and 20 µL multichannel pipettes (2 sets are recommended: 1 set for RNA handling [Reverse Transcription] and 1 set for DNA [qPCR])
- Pipette tips with filters intended for use with pipettes listed above
- Refrigerated microtube support for 1.5 mL, 0.2 mL microtubes or PCR plates
- Tube and plate Vortex
- 1.5 mL microtubes and 0.2 mL strip microcentrifuge
- 96 well plate centrifuge
- Thermocycler
- Real time PCR equipment
- RNase free single use gloves
- Cleaning agent for ADN/ARN
- Ethanol 70%
- Cleaning agent, RNase/DNase free (for periodic application)
- Capillary electrophoresis system for QC of RNA extraction (Example Fragment Analyzer) - Optional
- Reagents for running capillary electrophoresis

Additional notes:

- PCR instrument should:
 - Be designed for quantitative real-time PCR
 - Be equipped with a programmable thermic bloc
 - Have an excitation light: LEDs, lamp or laser compatible with FAM fluorophore
 - Have a filter set adapted to detection of FAM fluorescent reporter probes
 - Be connected to a computer with analysis software able to transfer data via a flat file format.

It is mandatory to ensure that all testing equipment and instruments used are duly installed, calibrated and checked according to supplier's instructions before testing.

6- Reagents

6.1- Reagent preparation

All reagents supplied with the miRpredX 31-3p kit are ready to use. Some reagents are required to be thawed before use. All reagents should be homogenized with vortex and centrifuged before use to ensure all solution is at the bottom of the tube.

All set-up should be performed at 4°C.

6.2- Storage and shelf life

Upon receipt, qPCR Master Mix should be removed from the kit box and stored refrigerated at 2°C to 8°C. If separate reagent batches are prepared, insure that different batches are not mixed when performing the test.

The remainder of the reagents in the miRpredX 31-3p kit should be stored in the original box at -15°C to -25°C up to final use in a freezer at that has no automated defrost program. Do not use reagents from the kit after the expiration date. IntegraGen cannot guarantee the accuracy of test results obtained from the use of the kit after its expiration date.

7- Precautions

None of the reagents included in the miRpredX 31-3p kit is harmful in the quantity supplied. A safety data sheet for the miRpredX 31-3p kit is available for download at: www.integragen.com/oncology/miRpredX-31-3p/documentation

The miRpredX 31-3p kit does not contain any substance of human or animal origin that could be infectious.

The miRpredX 31-3p Kit should only be handled by trained laboratory professionals who are familiar with the appropriate testing techniques. Always wear a laboratory coat, powder free and RNase free disposable gloves and protective goggles when using reagents from the kit and performing the pre-analytical and analytical steps associated with miR-31-3p expression testing (see Figure 5 above).

All test reagents and disposable supplies should be disposed of as biological waste in agreement with local regulations.

It is recommended that molecular biology grade water should be aliquoted in RNase free tubes before starting sample handling. Water used for the RT and qPCR steps should be from different aliquots. It is recommended to prepare enough aliquots according to the number of times kit will be used.

Prepared aliquots should be used only one time.

miRpredX 31-3p kit components can undergo up to 3 freeze/thaw cycles without affecting test performance. Do not use the miRpredX 31-3p kit beyond this limit of 3 freeze/thaw cycles.

8- Protocol

8.1- Process for testing for miR-31-3p expression

Quantification of miR-31-3p expression is performed via the following steps:

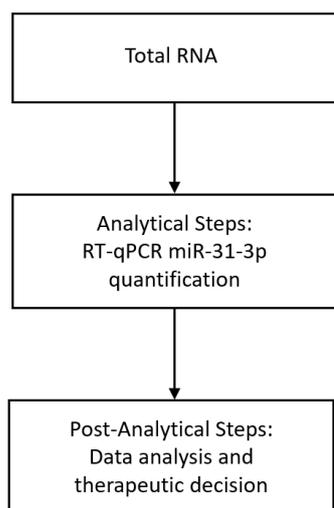


Figure 6: Total RNA is isolated from FFPE tumor tissue samples (surgery or biopsy). Reverse transcription is then performed and gene expression is measured by real-time PCR (RT-qPCR). For each sample ΔCt is calculated as the difference between the microRNA Calibrator Ct and miR-31-3p Ct compared to the mean value of ΔCt for the standard solution. RQ value = $2^{-\Delta\Delta Ct}$. The cut-off threshold for low expression of miR-31-3p is a RQ of 1.36. Patients with primary tumor samples with a RQ above 1.36 are considered high expressors.

8.2- Sampling and handling/storage of RNA samples

The miRpredX 31-3p kit designed to determine the expression of miR-31-3p in FFPE tissue obtained from tumor blocks (FFPE) from the primary tumors of RAS WT mCRC patients.¹³⁻¹⁵ All tissue samples should be considered as biological hazard.

To obtain test results that accurately represents the level of expression of RNA in a tumor, the sample should be extracted from the tumor area and not from surrounding healthy tissues. This sample should be obtained by scraping an identified tumor area on the slide with a scalpel. A new disposable scalpel should be used for each sample tested.

Insure extracted RNA complies with criteria listed in “Extraction acceptance criteria” below.

Recommendations:

Please refer to and closely follow manufacturer's recommendations included in the instructions for use which accompany the extraction kit used.

Use FFPE samples (slices, shavings or punch biopsies) containing at least 20% of tumor cells per microdissection area. Extract samples from tumor area only.

Use FFPE tissue quantity as recommended in extraction kit user's manual.

8.3- Extraction

The miRpredX 31-3p kit has been validated with the following extraction kits:

- *miRNeasy FFPE kit (Qiagen, Ref 217504)*
- *AllPrep DNA/RNA FFPE Kit (Qiagen, Ref 80234)*
- *QIAasymphony RNA kit (Qiagen 931636, FFPE protocol)*

Extract RNA according to the manufacturer's instructions for use which accompany the selected kit.

Utilized your laboratory's established method for quality control when extracting RNA from the tumor sample.

Maintain extracted RNA on ice or frozen before use. If extracted RNA is not to be analyzed immediately, store at -80°C.

Extraction acceptance criteria:

- *Follow RNA extraction kit recommendations for extracted RNA quality evaluation. Capillary electrophoretic methods are preferred.*
- *For electrophoretic RNA qualification, RNA migration profile should show a reasonable amount of small and microRNA (between 20 and 200 nt), with an average size of 500 to 1500 nt, while also containing large RNA (>3000 nt). (See Figure 7 and Figure 8 below).*
- *RNA Integrity Number (RIN) is not a good criterion to determine RNA quality following FFPE tissue extraction. Only a migration profile gives a good idea of fragment distribution after extraction.*

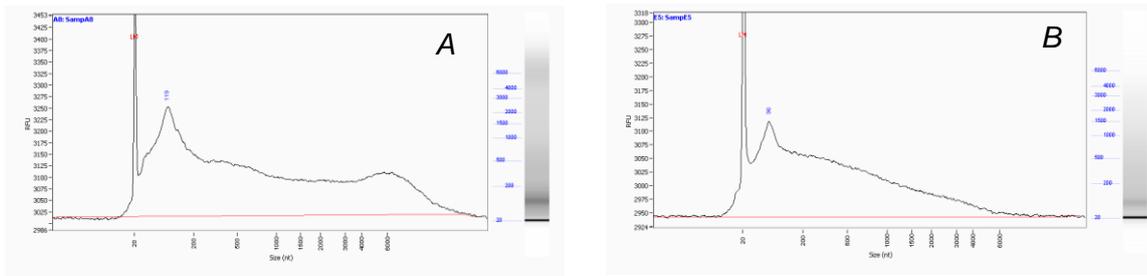


Figure 7: Examples of correct profiles for miR-31-3p expression analysis. Images from Fragment Analyzer® (Standard Sensitivity RNA Analysis Kit - DNF-489) after extraction with miRNeasy FFPE kit (Qiagen).

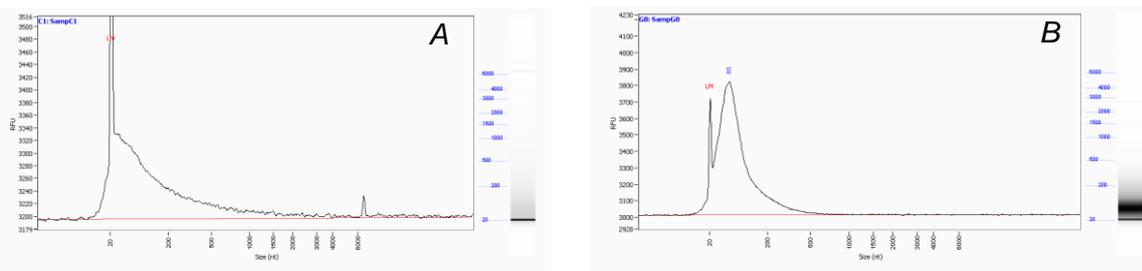


Figure 8: Example incorrect profile for miR-31-3p expression analysis. Profiles A and B show total RNA degradation profiles, lack of RNA >2000nt in A and lack of RNA >600nt in B. Images from Fragment Analyzer® (Standard Sensitivity RNA Analysis Kit - DNF-489, AATI) after extraction with miRNeasy FFPE kit (Qiagen).

8.4- Analytical steps

miR-31-3p expression is quantified from extracted RNAs in 2 technical steps (Reverse transcription followed by real time quantitative PCR).

The expression levels of each miRNA transcript (miR-31-3p and miR-calibrator) are measured with qPCR following extraction of total RNA from the FFPE sample obtained from the patient's tumor. Following specific transcription of the two microRNAs, miR-31-3p expression level is compared to the expression level the miR-calibrator for each sample and the difference is compared to the difference between the standard miR-31-3p and miR-calibrator solutions provided with the miRpredX 31-3p kit.

A standard curve is used to validate that both miR-31-3p and the miR-calibrator are measured in the linearity range of the standard curve. Relative concentrations remain unchanged over the dilution curve. The difference between miR-31-3p Ct and the miR-calibrator Ct for the reference standard S2 has been established as a reference value to calculate miR-31-3p expression levels for the samples being tested.

8.5- Post-analytical steps

Results are expressed in Ct representing the number of PCR cycles required to obtain a significant fluorescent signal in the exponential phase of reaction.

Raw data is transferred to calculation table. IntegraGen has developed a spreadsheet which can be used to run these calculations. This spreadsheet can be downloaded at: www.integragen.com/oncology/miRpredX-31-3p/documentation

For each sample, $RQ = 2^{-\Delta\Delta Ct}$ should be calculated via a prepared datasheet which is able to define if miR-31-3p expression level is low or high. The miR-31-3p expression level should be reported to the biologist or oncologist who ordered the test once results are obtained.

A recent study has validated a cut-off value of 1.36 which differentiates low miR-31-3p from high miR-31-3p expressors.² RAS WT mCRC patients whose tumors have a low expression of miR-31-3p ($RQ < 1.36$) have an improved response to anti-EGFR therapy when used in first-line when compared to anti-VEGF therapy. Low miR-31-3p expressors also have a better response to anti-EGFR therapy versus chemotherapy alone when used for second or further lines of treatment. RAS WT mCRC patients whose tumors have a high expression of miR-31-3p ($RQ \geq 1.36$) have a similar response to first-line anti-EGFR and anti-VEGF therapy. High miR-31-3p expressors also have a better response to chemotherapy alone versus anti-EGFR therapy when used for second or further lines of treatment.

9- Protocols

9.1- Sample preparation: Total RNA extraction

Reagents for RNA extraction kit are not supplied with the miRpredX kit. RNA extraction should be performed with a kit which has been validated for use with the miRpredX kit (see Section 8.3). Sample preparation should be performed according to manufacturer's instructions for the selected kit.

It is recommended that a minimum of 30 ng of total RNA be utilized to run the reverse transcription step.

Dilute the RNA in molecular biology grade water in order to get a normalized concentration of 12 ng/ μ L.

9.2- Analytical step

Note: The following information is for use with 96 well plates. The same steps can be performed in 0.2 mL tubes.

9.2.1- Reverse transcription

9.2.1. a) Plate layout

Calculate the total number of samples to be run including the 4 standards which will run to obtain the standard curve and NTC-RT.

Prepare RT Master Mix in a 1.5 mL sterile tube, including a 10% overage to cover pipetting losses.

Examples of reverse transcription (RT) plate layout for determination of miR-31-3p expression from 12 FFPE tumor samples:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1			#1	#5	#9						
B	S2			#2	#6	#10						
C	S3			#3	#7	#11						
D	S4			#4	#8	#12						
E				NTC								
F												
G												
H												

9.2.1. b) Plate set-up for RT

- Remove vials with standard reagents (S1, S2, S3, S4), NTP Premix A (RT1), and RT primers (PI and PN) from freezer and allow to thaw at 4°C.

Note: RT Premix B (RT2) should be thawed just prior to use.

- Once solutions are liquid, prepare Master Mix on ice.
- While reagents are being thawed, use the following table as a guide to prepare RT Master Mix (volumes in μL). Multiple the number of wells to be used for the Master Mix by the listed quantities. Include some overage to account for pipetting loss.

Reagent description	Tube code	Volume (μL)
miR-31-3p Primer	PI	1.5
miR-calibrator Primer	PN	1.5
NTP Premix A	RT1	1.65
RT Premix B	RT2	1.19
Standard, H ₂ O or sample	S1, S2, S3, S4 or L or ID#...	2.5
Nuclease, free-water	H2O	6.66

Table 3. Reverse Transcription Master Mix and RNA sample preparation.

- Vortex all reagents then centrifuge to have all reagents at bottom of tubes.
- Annotate a 1.5 mL tube to prepare Master Mix.
- Vortex Master Mix then centrifuge to have all reagents at bottom of tube.
- Maintain Master Mix on ice.
- Dispense 12.5 μL of Master Mix into each well to be used.
- Vortex and centrifuge each standard tube (S1, S2, S3, S4), patient samples and water aliquot. Dispense 2.5 μL of each standard, patient sample, and water into well already containing the

RT Master Mix. Use a new tip for each sample, NTC and standards to avoid any cross-contamination. Each well will contain 15µl. Maintain plate at 4°C.

- Seal the plate with appropriate sealant.
- Centrifuge plate at 1800 rpm for 30 seconds. Check that the solution is at the bottom of each well, if not, centrifuge again.
- Incubate plate at 4°C for at least 2 minutes.
- Start cycling program (see Table 2) and place plate in thermocycler as soon as the temperature has reached 16°C.

Temperature	Time
16°C	30 min
42°C	30 min
85°C	5 min
10°C	∞

Table 4. RT cycler program

- When finished store plate at 4°C if qPCR will run the same day, if not store properly identified plate at -20°C.

9.2.2- qPCR Step

For qPCR, repeat the above steps to prepare the two (2) Master Mixes:

1/ miR-31-3p

2/ miR-calibrator

9.2.2. a) Plate layout

Example of qPCR plate layout for 12 FFPE tumor samples (duplicate):

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	#1	#1	#7	#7	S1	S1	#1	#1	#7	#7
B	S2	S2	#2	#2	#8	#8	S2	S2	#2	#2	#8	#8
C	S3	S3	#3	#3	#9	#9	S3	S3	#3	#3	#9	#9
D	S4	S4	#4	#4	#10	#10	S4	S4	#4	#4	#10	#10
E	NTC-RT	NTC-RT	#5	#5	#11	#11	NTC-RT	NTC-RT	#5	#5	#11	#11
F	NTC-qPCR	NTC-qPCR	#6	#6	#12	#12	NTC-qPCR	NTC-qPCR	#6	#6	#12	#12
G												
H												
	miR-31-3p						miR-calibrator					

9.2.2. b) General Procedure

- Thaw reagent vials AI, AN, and H₂O at 4°C with qPCR MM.
- Vortex and centrifuge each tube before use.
- Prepare Master Mixes according to tables 6 and 7.

9.2.2. c) miR-31-3p Reaction (name I):

Reagent	Cap ID	Reaction volume
miR-31-3p Probe	AI	1.5 µL
RT product or H ₂ O (NTC)	ID#...	2.7 µL
qPCR Master Mix	qPCR MM	7.5 µL
Water, nuclease-free	H ₂ O	3.3 µL

Table 6: qPCR mix for miR-31-3p per well

9.2.2. d) Control Reaction (name N):

Reagent	Cap ID	Reaction volume
miR-calibrator Probe	AN	1.5 µL
RT product or H ₂ O (NTC)	ID#...	2.7 µL
qPCR Master Mix	qPCR MM	7.5 µL
Water, nuclease-free	H ₂ O	3.3 µL

Table 7: qPCR mix for Calibrator per well

- Vortex (10 seconds) and centrifuge each reagent mix.
- Dispense in each miR-31-3p used well of plate 12.3 µL of reaction mix.
- Proceed same way for reagent Mix N in calibrator wells.
- Vortex and centrifuge 30 seconds at 1800 rpm qPCR plate.
- Dispense 2.7 µL of each sample standard RT product in each destination well I and N that already contain 12.3 µL mix. Each well will contain a total of 15 µL.
- Seal PCR plate with appropriate sealant, vortex and centrifuge at 1800 rpm for 1 minute and place in qPCR machine.
- Start PCR machine according to following recommendations.

9.9.3. Specific qPCR equipment configuration

For general information for machine set-up please follow manufacturer recommendations and instructions for use.

For machines not described in this manual please contact technical support (seen § 14).

9.2.3. a) ABI 7900HT

In PCR system create your experiment and a plate layout with following parameters:

- Parameters

Instrument	7900HT (384 well)
Analysis mode	Standard Curve
Reaction volume	15 µL
Slope	Standard (default 100%)
Passive reference	ROX™
Reporter	FAM™
Quencher	None

Table 9: ABI 7900HT parameters

- Temperature profile and Reporter acquisition

	Step	Number of cycles	Mode of acquisition	Temperature (°C)	Time (min:sec)
Denaturation	Single	1	Off	95	10:00
Amplification	Cycles	40	Off	95	00:15
			On	60	01:00

Table 10: qPCR program

Collect data up to 40 cycles.

9.2.3. b) StepOnePlus

In PCR system create your experiment and a plate layout with following parameters:

- Parameters

Instrument	StepOnePlus (96 wells)
Analysis mode	Quantitation
Quantitation	Std Curve
Reaction volume	15 µL
Slope	Standard (default)
Passive reference	ROX™
Reporter	FAM™
Quencher	None

Table 11: StepOnePlus parameters

- **Temperature profile and Reporter acquisition**

	Step	Number of cycles	Mode of acquisition	Temperature (°C)	Time (min:sec)
<i>Denaturation</i>	<i>Single</i>	<i>1</i>	<i>Off</i>	<i>95</i>	<i>10:00</i>
<i>Amplification</i>	<i>Cycles</i>	<i>40</i>	<i>Off</i>	<i>95</i>	<i>00:15</i>
			<i>On</i>	<i>60</i>	<i>01:00</i>

Table 12: qPCR program

Collect data up to 40 cycles.

9.2.3. c) QuantStudio 5

In PCR system create your experiment and a plate layout with following parameters:

- **Parameters**

<i>Instrument</i>	<i>QuantStudio 5 (96 wells)</i>
<i>Analysis mode</i>	<i>Standard Curve</i>
<i>Reaction volume</i>	<i>15 µL</i>
<i>Slope</i>	<i>Mode standard (with ramp rates of 1,6°C/s)</i>
<i>Passive reference</i>	<i>ROX™</i>
<i>Reporter</i>	<i>FAM™</i>
<i>Quencher</i>	<i>None</i>

Table 13: Quant Studio 5 parameters

- **Temperature profile and Reporter acquisition**

	Step	Number of cycles	Mode of acquisition	Temperature (°C)	Time (min:sec)
<i>Denaturation</i>	<i>Single</i>	<i>1</i>	<i>Off</i>	<i>95</i>	<i>10:00</i>
<i>Amplification</i>	<i>Cycles</i>	<i>40</i>	<i>Off</i>	<i>95</i>	<i>00:15</i>
			<i>On</i>	<i>60</i>	<i>01:00</i>

Table 14: qPCR program

Collect data up to 40 cycles.

9.2.3. d) LightCycler® 480 Instrument II

In PCR system create your experiment and a plate layout with following parameters:

- Parameters

Instrument	LC480 II (96 wells)
Analysis mode	Absolute Quantitation
Detection forma	Dual Color Hydrolysis Probe / UPL Probe
Reaction volume	15 µL
Slope	1,6°C/s
Reporter	FAM™
Quencher	None

Table 15: Quant Studio 5 parameters

Make sure that in Customize field, « Filter Combination » is FAM™ (465-510).

- Temperature profile and Reporter acquisition

	Step	Number of cycles	Mode of acquisition	Temperature (°C)	Time (min:sec)
Denaturation	Single	1	Off	95	10:00
Amplification	Cycles	40	Off	95	00:15
			On	60	01:00

Table 16: qPCR program

Collect data up to 40 cycles.

9.3- Post analysis

Before data analysis visualize amplification graphics.

9.3.1- Analysis with 7900HT, StepOnePlus and QuantStudio 5 (ABI)

Use the following parameters:

- In qPCR software, leave automatic mode as the base line
- Manually configure threshold at 0.2 for both miR-31-3p and miR-calibrator (Y axis is logarithmic scale)

Confirm set-up for software to update results.

9.3.2- Analysis with LC480 II (Roche)

Use second derivative method. Base line and threshold are set automatically.

Confirm set-up for software to update results.

9.3.3- IntegraGen spreadsheet analysis

Enter Ct values of standards followed by each sample's Ct values. The spreadsheet will automatically calculate the patient's miR-31-3p expression status (e.g. high or low expressor).

Microsoft Excel spreadsheet and an OpenOffice spreadsheet can be downloaded at:

www.integragen.com/oncology/miRpredX-31-3p/documentation

9.3.4- Free data analysis

Export and analysis can be performed with your method of choice following the recommendation(s) provided in the instrument's user manual.

10- Criteria of acceptance

10.1- Standards

- Standard curve slope should be between -3.92 and -2.92 (inclusive).
- r^2 of each curve should be between 0.95 and 1.00 (inclusive), and better if $r^2 \geq 0.98$.
- ΔCt between miR-31-3p and miR-calibrator standard curves should be 2.8 ± 0.5 .
- Ct at S2 Standard for miR-calibrator should be between 20 and 24 (inclusive).

10.2- Negative control

The negative control should not show any detectable fluorescence. If fluorescence is detected and cut the threshold before the cycle n°39, repeat the assay.

10.3- ΔCt reproducibility between replicates at reference Standard S2

The ΔCt between the miR-calibrator and miR-31-3p of replicates of S2 should not exceed 0.5 Ct. If criteria are not reached analysis should be repeated.

All criteria are summarized in following table.

Acceptance criteria	Acceptance value
Ct difference between replicates at S2 standard	$\leq 0.5Ct$
Value of ΔCt at S2 standard	2.80 ± 0.5
Standard curves slopes	-3.92 to -2.92 (inclusive)
Standard curves r^2	> 0.95 , better if > 0.98
NTC RT and qPCR	Ct > 39 or "undetermined"

Table 17: Acceptance criteria for qPCR

11- Data analysis and interpretation

11.1- RQ Calculation

Set standard curve and check its compliance:

11.1. 1- Standard (Reference) calculation

- For S2, report miR-31-3p Ct data. Calculate mean value: $Ct_{miRNA Std}$
- For S2, report miR-calibrator Ct. Calculate mean value: $Ct_{calibrator Std}$
- Calculate difference: $Ct_{miRNA Std} - Ct_{calibrator Std} = \Delta Ct_{Std}$

11.1. 2- Sample Normalization

- For each sample, report miR-31-3p Ct data. Calculate mean value: $Ct_{miRNA Sample}$
- For S2, report miR-calibrator Ct. Calculate mean value: $Ct_{calibrator Sample}$
- Calculate difference: $Ct_{miRNA Sample} - Ct_{calibrator Sample} = \Delta Ct_{Sample}$

11.1. 3- Normalization with Standard

- Calculate $(\Delta Ct_{Sample} - \Delta Ct_{Std}) - K^* = \Delta \Delta Ct$

11.1. 4- RQ value calculation

- $RQ = 2^{-\Delta \Delta Ct}$

*The analytical factor for the standard (1.43 for batches #IG170801 to #IG181204)

11.2- Sample acceptance criteria

- Ct for calibrator should be ≤ 27 .
 - **Alert level**

At Ct > 27: for these samples results should be checked carefully specially if close to cut-off value.

➤ **Rejected level**

At Ct > 29: Any sample with miR-calibrator Ct above 29 should be extracted and re-analyzed.

- ΔCt difference between 2 replicates of same samples should be ≤ 0.5 .
- For all samples where replicates values for ΔCt is > 0.5:
 - If both samples RQ is < 1.36 and mean RQ < 0.96, miR-31-3p expression level is low.
 - If both samples RQ is ≥ 1.36 and mean RQ ≥ 1.92 , expression level is high.
 - In all other cases: mean RQ between 0.96 and 1.92 or if one replicate is above 1.36 and the other replicate below 1.36, repeat the assay.
- Uncommon amplification curve
If one sample has a non-typical amplification curve, it should be re-tested.

11.3- Classification rules

For samples complying with all criteria (§11.1)

- If both replicates have $RQ < 1.36$ and mean $RQ < 1.25$, level of expression of miR-31-3p is low.
- If both replicates have $RQ \geq 1.36$ and mean $RQ \geq 1.47$, level of expression of miR-31-3p is high.
- If mean RQ is between 1.25 (excluded) and 1.47 (included) result is in uncertainty measurement area, biologist will decide or not to repeat measurement to define final status.
- For samples with ΔCt difference > 0.5 but with consistent RQ values for replicate (both $RQ < 1.36$ or both $RQ \geq 1.36$):
 - If mean $RQ < 0.96$ level of expression of miR-31-3p is low.
 - If mean $RQ \geq 1.92$ level of expression of miR-31-3p is high.

Decision tree is described here after:

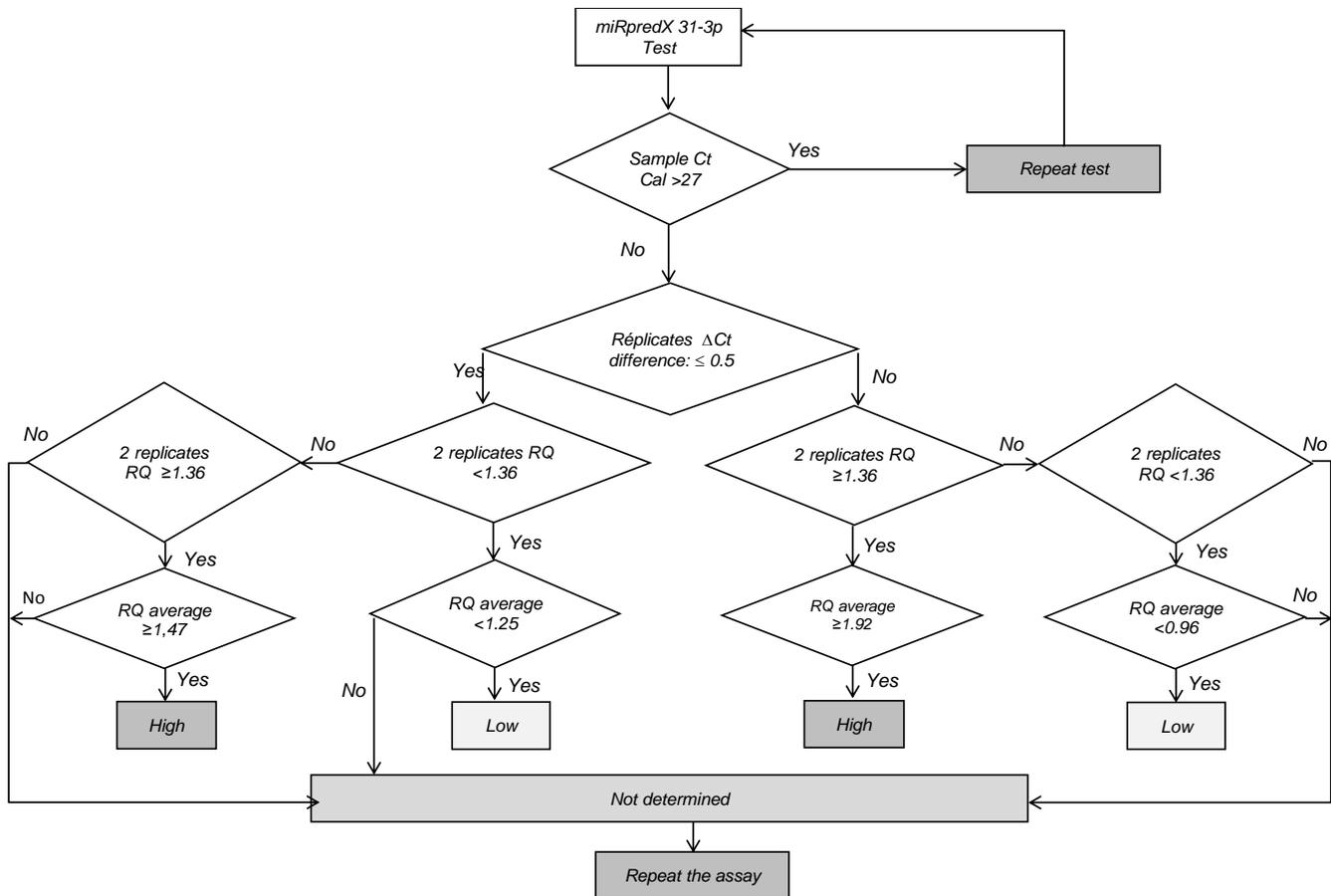


Figure 9: Decision tree for tumor samples classification

11.4- General recommendations for miR-31-3p quantification

To perform successful miR-31-3p quantification, results should comply with criteria defined in chapter “10- Extraction acceptance criteria”.

In the case of acceptance criteria are not met for all samples, all samples should be re-analyzed.

It is acceptable to remove one of the dilution results for the standard curve if it is out of range. The decision to remove this result should be recorded in analytical report.

Biologist should follow step-by-step instructions for data calculation using an Excel spreadsheet and recheck inserted data. The Excel spreadsheet will calculate automatically patient status, color codes help in data interpretation.

The cell containing information for a patient sample will turn green or blue depending on the calculated RQ results:

- If the RQ for the patient sample is lower than cut-off: cell will be green with mention "LOW".*
- If the RQ for the patient sample is higher or equal than cut-off: cell will be blue with mention "HIGH".*

The cell for a patient's sample will turn yellow when:*

- Mean miR-calibrator Ct is > 27*

The cell for a patient sample will turn orange when:*

- Mean miR-calibrator Ct is > 29*

** Refer to §11.2 for interpretation*

Note: IntegraGen offers customers an Excel spreadsheet (www.integragen.com/oncology/miRpredX-31-3p/documentation) which calculates the expression level of miR-31-3p independently of Standard curve validation or samples validation (refer to §10. and §11.). Automated analysis is given only as preliminary result. The only valid analysis is the one performed by biologist after checking all acceptance criteria. IntegraGen is not liable for a clinical decision based only on automated calculation of Excel spreadsheet.

11.5- Data interpretation

- RAS WT mCRC patients whose tumors have a low expression of miR-31-3p ($RQ < 1.36$) have an improved response to anti-EGFR therapy when used in first-line when compared to anti-VEGF therapy. Low miR-31-3p expressers also have a better response to anti-EGFR therapy versus chemotherapy alone when used for second or further lines of treatment.*
- RAS WT mCRC patients whose tumors have a high expression of miR-31-3p ($RQ \geq 1.36$) have a similar response to first-line anti-EGFR and anti-VEGF therapy. High miR-31-3p expressers also have a better response to chemotherapy alone versus anti-EGFR therapy when used for second or further lines of treatment.*
- Result will be "NOT DEFINED" if no value is issued by the test or if acceptance criteria are not met (negative control positive, $r^2 < 0.95$, standards ΔCt / slopes not valid).*

12- Features and performance

Details related to performance validation are published in technical paper. ¹⁷

12.1- Cut-off validation

The cut-off value which differentiates low and high miR-31-3p expressors is based on the results from a prospective-retrospective clinical study of 340 RAS WT patients with mCRC which validated this cut-off.²

12.2- Limit of detection

Standard was diluted down to the point of lack of amplification. The lowest significant measurement of miR-31-3p is 0.005ng of standard total RNA.

12.3- Standard curve linearity range

Linearity was measured with 12 independent analyses of standard curves.

- Average correlation rate for miR-31-3p is: $r^2 = 0.999$
- Average correlation rate for calibrator is: $r^2 = 0.999$

12.4- Δ Ct reproducibility for calculation reference standard

The reproducibility of Δ Ct at standard S2 was measured with 14 independent analyses. Average Δ Ct at S2 is 2.80. Standard deviation is 0.17.

	Mean (Δ Ct)	SD (Δ Ct)	CV
Standard S2	2.8	0.17	6%

Figure 18: Δ Ct and Standard S2 reproducibility

12.5- Intra-assay variability

Intra-assay variation is measured using the same PCR plate with 16 replicates of a low expression sample and 16 replicates of high expression level sample quantified as described in the miRpredX 31-3p Instructions for Use.

Sample	N	$\Delta\Delta$ Ct			RQ		
		Average	SD	CV	Average	SD	CV
E1 (Low)	16	2.92	0.29	9.93%	0.13	0.03	23.07%
E2 (High)	16	-2.35	0.08	3.40%	5.10	0.29	5.68%

Figure 19: Results of operator 1 at Day 0

12.6- Inter-assay variability

Inter-assay variation is measured with 16 replicates of a low expression sample and 15 replicates of high expression level sample in 2 independent platforms with 2 different operators at different days quantified as described in the miRpredX 31-3p Instructions for Use.

Sample	N	$\Delta\Delta Ct$		RQ	
		Average	SD	Average	SD
E1 (Low)	16	3.03	0.22	0.12	0.02
E2 (High)	15	-2.31	0.14	4.83	0.27

Figure 20: Results of operator 2 à Day 1

Bias between 2 sets of analysis is evaluated with Bland-Altman statistical method (16).

Sample	Bias $\Delta\Delta Ct$	Bias RQ
E1 (Low)	0.1178	-0.0114
E2 (High)	0.0355	-0.1107

Figure 21: Bias measurement with Bland Altman statistical analysis

Bias between the 2 sets of measurement is lower than standard deviation showing no inter-assay variability.

12.7- Accuracy

Precision was evaluated with the standard addition method. A known quantity of miR-31-3p was added to a known low content RNA sample.

Assay confirms a x10 rate between sample with 1500 copies miR-31-3p added and sample with 150 copies miR-31-3p added.

Method is accurate.

12.8- Robustness

Temperature: RT reaction was performed with a standard curve at 59°C, 60°C and 61°C. Method is robust in the range of temperature.

PCR Master Mix: PCR was performed with a standard curve with 80% of Master Mix. Method is robust with 80% of PCR Master Mix.

PCR was performed with a standard curve with Master Mix stored at different temperatures: -20°C, 4°C and at room temperature. Method is robust with the 3 tested temperatures for Master Mix storage conditions.

12.9- Specificity

Specificity was evaluated with the standard addition method. A known quantity of miR-31-5p, a microRNA co-transcribed with miR-31-3p, was added to a known low content RNA sample.

Results show lack of cross detection between miR-31-3p and miR-31-5p.

12.10- Kits limitation

Contaminants carried over during extraction of paraffinized samples may interfere with Reverse transcription or PCR amplification.

This kit is validated for FFPE tissue obtained from tumor blocks or biopsies from patients with mCRC. It is not validated for other cancer or sample types.

Please refer to the Troubleshooting section of this document (Chapter 13) to obtain assistance to solve problems related to non-compliant results interpretation or to obtain more details on factors that could affect test performance.

As the testing method used with the miRpredX 31-3p kit is highly sensitive, please be advised to use caution and follow the below recommendations at all times:

- Handle samples with care to avoid any potential cross contamination.
- Work in a pre-PCR area or a place adapted to prepare Reverse Transcription. Proceed to full decontamination after each test step.
- Use different pipet sets for the RT and PCR steps.
- Take care when pipetting into 96 well plates to not contaminate adjacent wells during mixing. Change tips anytime a different reagent or samples is handled.
- Keep solutions and mixes at 4°C during setup.

13- Trouble shooting guide

13.1- Out of specification Standards

Non-Compliance	Possible Cause	Solution
Low level of fluorescence at plateau.	Low reagent content; Reagents degradation.	Check Master mix calculations; Check for pipetting accuracy. Repeat assay with fresh prepared reagents.
	Pipetting mistake.	Repeat the assay.
	Threshold set-up.	Contact tech support: support-miRpredX@integrangen.com

<i>Atypical PCR curve.</i>	<i>Mechanical or plasticware problem.</i>	<i>Contact your PCR machine support or your plasticware supplier.</i>
	<i>Low amplification or low fluorescence.</i>	<i>Repeat assay with careful pipetting.</i>
<i>Ct or ΔCt > 0.5 at S2 Standard.</i>	<i>Pipetting mistake.</i>	<i>Check pipet calibration; Keep pipet vertical when pipetting.</i>
	<i>Lack of reagents homogeneity.</i>	<i>Mix reagents carefully and pipet cDNA with care for PCR reaction.</i>
	<i>High Ct: > 34.</i>	<i>See § 11.1- and 11.2-.</i>
<i>ΔCt for S2 is out of specification.</i>	<i>Protocol for RT-qPCR was not followed with care.</i>	<i>Repeat RT-qPCR Contact technical support: support-miRpredX@integrage.com</i>
<i>r² < 0.95</i>	<i>Pipetting mistake.</i>	<i>It is allowed to exclude <u>only</u> one of the standard to reach acceptance criteria.</i>
<i>Standard curve slope is out of specifications.</i>	<i>RT mix is not prepared at 4°C.</i>	<i>Repeat the full process.</i>
	<i>RT plate is not prepared at 4°C.</i>	
	<i>Pipetting mistake in RT.</i>	
	<i>Pipetting mistake in qPCR.</i>	

13.2- Contaminated NTC

Non-Compliance	Possible Cause	Solution
<i>NTC amplification.</i>	<i>Plasticware or solutions are contaminated.</i>	<i>Use RNA/DNA, RNase/DNase free plastic. Prepare fresh solution or use new reagents in new plastic batch.</i>

	<i>Working area contamination.</i>	<i>Clean working area with 70% ethanol or ADN / ARN and RNase/DNase removal solution.</i>
	<i>Wrong pipetting method.</i>	<i>Take care while pipetting to avoid spilling and change pipet tip for each sample.</i>
	<i>Plates are not sealed properly.</i>	<i>Seal RT and qPCR plate with care.</i>

13.3- Lack of amplification- no fluorescence is detected

Non-Compliance	Possible Cause	Solution
<i>No signal is detected for standard curve and samples.</i>	<i>Fluorophore is degraded.</i>	<i>Store probes protected from light.</i>
	<i>Equipment not properly calibrated for the fluorophore.</i>	<i>Check calibration.</i>
	<i>Wrong machine set-up.</i>	<i>Check instrument has correct set-up for the fluorophore.</i>
	<i>RT did not worked.</i>	<i>Repeat RT.</i>
	<i>Missing reagent in RT/PCR. cDNA not pipetted in qPCR plate.</i>	<i>Repeat RT and PCR.</i>
	<i>Incorrect Thermocycler program.</i>	<i>Check program and temperature.</i>
	<i>Wrong block temperature.</i>	<i>Perform a calibration for block temperature.</i>
	<i>Wrong selection of well position during analysis.</i>	<i>Cross check plate layout and data analysis.</i>

13.4- Interpretation

Non-Compliance	Possible Cause	Solution
<p><i>Regular and compliant level of calibrator expression and lack of miR-31-3p expression.</i></p>	<p><i>Low miR-31-3p expression.</i></p>	<p><i>1/ Repeat RNA quantification.</i></p> <p><i>2/ Repeat the assay.</i></p> <p><i>3/ Repeat the assay with 60ng of total RNA to confirm results.</i></p>
<p><i>miR-calibrator Ct > 27 and ≤ 31.</i></p>	<p><i>Low RNA extraction yield.</i></p>	<p><i>If Calibrator is consistent in analysis set and miR-31-3p is quantified, measure is compliant.</i></p> <p><i>If miR-31-3p is not detected:</i></p> <p><i>1/ Repeat RNA quantification.</i></p> <p><i>2/ Repeat the assay.</i></p> <p><i>3/ Repeat the assay with 60ng of total RNA to confirm results.</i></p>
<p><i>miR-calibrator Ct > 31</i></p>	<p><i>Bad sample quality, low RNA in RT, normalization mistake.</i></p>	<p><i>Repeat RNA extraction and quantification.</i></p> <p><i>Contact technical support: support-mirpredX@integragen.com</i></p>
<p><i>Technical replicates have difference between Ct > 0.5 cycles.</i></p>	<p><i>High technical variability.</i></p> <p><i>Low miR-31-3p expression level.</i></p>	<p><i>Follow recommendations of § 11, for any additional support contact support-miRpredX@integragen.com</i></p>

14- Quality Control

Following IntegraGen ISO13485:2016-certified Quality Management system requirements, each batch of miRpredX 31-3p is tested according to specifications before release.

Certificates of Analysis are available at: www.integragen.com/mirpredx-31-3p/certificates, or upon request at: support-miRpredX@integragen.com

15- Safety Information

Safety data sheet is available at www.integragen.com/oncology/miRpredX-31-3p/documentation

16-Technical support

IntegraGen technical support is available Monday through Friday (except holidays) from 9h to 12h30 and from 14h to 17h30 Central European Time (C.E.T.)

Contact information:

E-mail: support-miRpredX@integragen.com

Telephone: +33 (0)1 60 91 09 00

17- Vigilance

Please notify IntegraGen and local health authorities in case of an accident linked to the use of the miRpredX 31-3p kit.

Téléphone : + 33(0)1 60 91 09 00

Mail : contactq@integragen.com

18- Information

18.1- Manufacturer's data

	<p>IntegraGen SA Génopôle Campus 1 – Bât. 8 Porte n°840 5, Rue de Henri Desbruères 91030 Evry, France miRpredX@integragen.com support-miRpredX@integragen.com T : +33 (0)1 60 90 09 10</p>
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19- Symbols significance

	REFERENCE
	NUMBER OF REACTIONS
	STORAGE TEMPERATURE
	MANUFACTURER
	EXP.DATE
	BATCH NUMBER

20- Licenses, brands

miRpredX[®] and the miRpredX[®] logo are trademarks of IntegraGen S.A. The miRpredX 31-3p kit is covered by patent number EP2012/073535. IntegraGen S.A. holds the exclusive worldwide license for the sale of products associated with this patent.

ABI 7900HT[®], ABI StepOne+[®], ABI QS5[®] (Applied Biosystems); FAM[™], ROX[™] (Life Technologies); LightCycler[®] (Roche); Fragment Analyser[®], miRNeasy[®], QIASymphony[®] (QIAGEN): Brand names cited in the present document are protected names.

The miRpredX 31-3p kit is an in vitro Diagnostic kit, CE marked in compliance with European Directive98/79/CE for IVD Diagnostic kits.

USER INFORMATION: LICENCE LIMITS

The MGB Probe contained in this product is covered by applicable U.S. patents and corresponding patents outside the U.S. and is sold under a license from the ELITech Group. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use solely in the human in vitro diagnostic field for the medical management of patients (in accordance with applicable FDA and other regulatory requirements) and may not be used for any other commercial use, including without limitation repackaging or resale in any form. No right under any other patent claim or for any other use is conveyed expressly, by implication, or by estoppel. Corresponding products conveying rights for use in all other fields may be obtained from Life Technologies under a separate catalog number. For information on obtaining additional rights, please contact outlicensing@thermofisher.com or Out Licensing, Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, California 92008.

21- Product Ordering Information

Catalog #	Description	# of tests per kit
<i>IG-500-001</i>	<i>miRpredX 31-3p</i>	<i>12</i>

To order, call +33 (0)1 60 910 900 or send an e-mail miRpredX@integragen.com

The miRpredX 31-3p test kit has received IVD CE Mark approval and is commercially available in countries recognizing the CE Mark, or with applicable health authority registrations. The miRpredX 31-3p test kit is not available for sale in the United States.

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