# miRpredX 31-3p

*For in vitro diagnostic use only.*

## QUICK START GUIDE

CE IVD

∑ 12

REF IG-500-001
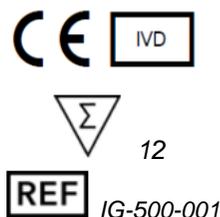
## 1- NOTE

*Please read protocol carefully before using the kit for the first time. An electronic version on the complete miRpredX Instructions for Use is available at www.integragen.com/oncology/miRpreX-31-3p/documentation.*

Results from the miRpredX 31-3p test are intended to support therapeutic decisions for RAS wild-type (WT) metastatic colorectal cancer (mCRC) patients.

The miRpredX 31-3p test predicts the potential clinical benefits associated with first-line anti-EGFR therapy compared to anti-VEGF therapy or when second or further lines of treatment with anti-EGFR therapy is beneficial versus chemotherapy alone for patients with RAS WT mCRC.

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.

## 2- DESCRIPTION

The miRpredX 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. The microRNA calibrator is a housekeeping miR gene which is not affected by the pathology and is utilized to normalize the assay and reduce sample preparation associated bias.

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

miR-specific standard curves are used to calculate measurement linearity of RT-QPCR and to quantify relative quantities of miR-31-3p and the microRNA calibrator in samples. Correlation coefficient ($r^2$) and the slope of the standard curve is used as criteria for the validity of test results.

The difference between miR-31-3p Ct and the microRNA calibrator Ct for each sample is compared with $\Delta Ct$ between both of the above and a standard point. $\Delta\Delta Ct$ is used to calculate relative level of expression RQ, calculated as RQ = $2^{-\Delta\Delta Ct}$.

## 3- KIT CONTENT

The miRpredX 31-3p Kit contains sufficient reagents for the measurement of miR-31-3p expression levels from twelve (12) FFPE tumor samples.

## 4- STORAGE

Upon receipt, the qPCR Master Mix should be stored at 4°C to 8°C. Other kit reagents should be stored between -15°C and -25°C.

If appropriately stored and unopened, miRpredX 31-3p reagents are stable for 18 months from the date of manufacture. IntegraGen does not recommend using the reagents beyond the validity date. Important: Reagents should be stored frozen until use. Reagents in the miRpredX 31-3p kit can be thawed and frozen up to 3 times.

## 5- RNA EXTRACTION

The miRpredX 31-3p kit is designed for the analysis of FFPE tumor samples obtained from the primary tumors of patients with RAS WT metastatic colorectal cancer (slices, punch biopsies).

Total RNA can be extracted with any RNA extraction kit that enables the extraction of microRNA. It is important to carefully follow the manufacturer's instructions included with the extraction kit.

Quantify total RNA after extraction before running RT. The recommended total RNA for running RT is 30 ng.

## 6- REVERSE TRANSCRIPTION

Prepare master mix according to following table for each reaction. It is recommended to include an extra quantity to prevent loss of material during pipetting.

|  | Reagent Tube code | Volume (µL) |
|---|---|---|
| miR-31-3p Primer | PI | 1.5 |
| miR-calibrator Primer | PN | 1.5 |
| RT Premix A | RT1 | 1.65 |
| NTP Premix B | RT2 | 1.19 |
| Nuclease, free-nuclease | H2O | 6.66 |

Dispense first, in each well to be used 12.5 µL of master mix. Then pipet 2.5 µL of each standard (S1, S2, S3, S4), tumor sample (ID#..., all at 12 ng/µL) and DNAse/RNAse free water (H2O) in their respective wells.

Run the reverse transcription step in a thermocycler according to following:

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

## 7- qPCR

*Run real-time PCR according to following table. It is important to include extra quantities to prevent pipetting losses.*

*miR-31-3p master mix:*

|  | Tube code | Volume (µL) |
|---|---|---|
| **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** | H2O | 3.30 µL |

*microRNA Calibrator master mix:*

|  | Tube code | Volume (µL) |
|---|---|---|
| **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** | H2O | 3.30 µL |

*Dispense 12.3 µL of each master mix in 2 wells for each tube according to plate design.*

*Pipet 2.7 µL of RT product in each of its respective wells containing miR-31-3p and microRNA calibrator master mix*

*Run real-time PCR with parameters describes in complete instructions for: 7900HT (ABI), StepOne+ (ABI), QuantSudio 5 (ABI) and LC480 (Roche). For any specific qPCR machine please contact technical support.*

- *Cycling program:*

| Number of cycles | Temperature | Time |
|---|---|---|
| 1 | 95°C | 10 minutes |
| 40 | 95°C | 15 seconds |
| 40 | 60°C | 1 minute |

**8- TEST VALIDATION CRITERIA**

*Standard curve should meet the following criteria to guarantee valid test results:*

| Curve acceptance criteria | Acceptance value |
|---|---|
| *Ct difference between replicates at S2 standard* | $\leq 0.5Ct$ |
| *$\Delta$Ct between biomarker and calibrator curve* | *2.8 ± 0.5* |
| *Standard curves slopes* | *-3.92< > -2.92* |
| *Standard curves R$^2$* | *At least > 0.95 better if > 0.98* |
| *NTC* | *Ct > 39* |

*All samples should be re-analyzed if any one of the above criteria is not met.*

**9- INTERPRETATION**

*$\Delta\Delta$Ct is calculated as:*

**[(Ct miR31-3p - Ct miRCal) sample] – [(CtmiR31-3p - CtmiRCal)Std – 1.43]**

**Rq is calculated as RQ = 2$^{-\Delta\Delta Ct}$.**

**Assay cut-off is RQ = 1.36.**

*Excel spread sheet for calculation can be downloaded at: www.integragen.com/oncology/miRpredX-31-3p/documentation.*

*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 ≥ 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.*

**10- TROUBLESHOOTING**

*A comprehensive troubleshooting guide along with frequently asked questions are available in Section 13 of the complete miRpredX 31-3p kit Instructions for Use which are available online at: www.integragen.com/oncology/miRpreX-31-3p/documentation.*

*Please contact us at support-miRpredX@integragen.com for additional support.*

*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.*

*For professional use only.*

**INTEGRAGEN**

*IntegraGen SA*
*Génopole Campus 1 – Bât. 8*
*5, Rue de Henri Desbruères*
*91000 Evry, FRANCE*

**www.integragen.com**

*July 2018 (Rev.5)*