Instructions for Use

RealLine KRAS Detect

KIT FOR THE QUALITATIVE DETECTION AND DIFFERENTIATION OF 7 MUTATIONS IN THE KRAS GENE BY REAL-TIME PCR

Research Use Only (RUO)

RealLine KRAS Detect	**REF MED20401**	**12 Tests**
	REF MED20410	**36 Tests**
valid from:	**April 2020**	

RealLine KRAS Detect

Explanation of symbols used in labeling:

RUO	For Research use only
LOT	Batch code
REF	Catalogue number
	Contains sufficient for <n> tests
	Use-by-date
	Temperature limit
	Consult instructions for use
	Keep away from sunlight
	Manufacturer

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This kit is validated for the real-time PCR cyclers:

- **IQ™5 iCycler (Bio-Rad)**
- **CFX96™ (Bio-Rad)**
- **RealLine Cycler (BIORON Diagnostics GmbH)**
- **LightCycler™ Nano (Roche)**

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1. CLINICAL INFORMATION

Colorectal cancer is the fourth most common cancer among men and women. Erbitux (Cetuximab) and Vectibix (Panitumumab) are drugs based on anti-EGFR antibodies that are used for targeted therapy of metastatic colorectal cancer (mCRC). About 40% of mCRC patients have mutations in the KRAS gene in the tumor. This tumors with KRAS mutations in codons 12 or 13 are resistant to anti-EGFR therapy [1,2], and the patients have no benefit. Accordingly, before the treatment of the patient with mCRC using Erbitux® or Vectibix® - the an anti-EGFR therapy - it is recommended to test the KRAS mutation status and to apply only to patients without such mutations [3, 4, 5].

2. INTENDED USE

The kit is designed to detect seven mutations of the codons 12 and 13 of the KRAS gene that are associated with resistance to anti-EGFR therapy (Table 1).

Table 1. KRAS gene mutations that can be detected by **RealLine KRAS Detect** Kit.

PCR mix number	Mutation, amino acid	Mutant codon, nucleotides*
1	Control mix	No mutation
2	G12C	T<u>G</u>T
3	G12S	A<u>G</u>T
4	G12R	C<u>G</u>T
5	G12V	G<u>T</u>T
6	G12D	G<u>A</u>T
7	G12A	G<u>C</u>T
8	G13D	G<u>A</u>C

** mutated nucleotide is underlined*

The mutations are detected by allele specific real-time PCR. With this kit no post-PCR processing is needed and the risk of contamination is minimized, as detection is performed in the same sealed tube without any further handling steps.

For the extraction of DNA from formalin-fixes tissue (FFPE-tissue), the kit is validated with:

- RealLine FFPET DNA Extraction Mag (REF MED32501, MED32503)
- RealLine FFPET DNA Extraction (MED20301)

- The kit is adapted for use on the real-time PCR devices: iQ™5 iCycler, CFX96™ (Bio-Rad Laboratories, Inc., USA), RealLine Cycler (BIORON Diagnostics GmbH), LightCycler™ Nano (Roche Diagnostics GmbH, Germany).

<u>Note:</u> The use of other instruments, other extraction kits or other volumes has to be validated by the user in the lab.

3. CONTENT OF THE KIT

The **RealLine KRAS Detect Kit** contains reagents for 12 reactions (REF MED20401) or 36 reactions (REF MED20410) for each mutation with unknown samples and controls. The kit contains colour coded tubes: Table 2.

Tube №	Color cap	Content	MED20401	MED20410
1	Green	Control PCR mix	740 µl	2200 µl
2	Yellow	PCR mix G12C	280 µl	840 µl
3	Yellow	PCR mix G12S	280 µl	840 µl
4	Yellow	PCR mix G12R	280 µl	840 µl
5	Yellow	PCR mix G12V	280 µl	840 µl
6	Yellow	PCR mix G12D	280 µl	840 µl
7	Yellow	PCR mix G12A	280 µl	840 µl
8	Yellow	PCR mix G13D	280 µl	840 µl
9	Blue	Taq DNA Polymerase	30 µl	90 µl
10	Red	Positive DNA control	105 µl	320 µl
11	White	PCR grade water	1.5ml	4ml

The label at the outer box does provide the information of **"Quality control chart (QC)"** with the relevant numbers for the evaluation of the data.

4. SUITABLE SAMPLE MATERIAL

DNA extracted from fresh, fresh-frozen or formalin fixed paraffin embedded (FFPE) tumor tissue section can be tested.

Tissue for DNA extraction should be verified by pathologist and should contain not less than 20% of tumor cells. About 2000 copies of the KRAS gene (6 ng of genomic DNA) are sufficient to perform PCR. Under such conditions, DNA containing more than 5% of mutant copies of the KRAS gene yield positive result in the test.

The Kit does not provide reagents for DNA isolation. For the extraction of the DNA the use of the RealLine FFPE DNA Extraction Kit (BIORON Diagnostics GmbH, REF MED20301) or RealLine FFPE DNA Extraction Mag Kit (BIORON Diagnostics GmbH, REF MED32501) is recommended.

5. PRODUCT USE LIMITATIONS

For Research Use Only.

Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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6. PRINCIPLE OF THE KIT

The kit includes **8** different PCR mastermixes:

- 7 mixes specific for 7 different mutations (Table 1)
- 1 Control PCR mix.

The products of the PCR with the **RealLine KRAS Detect** Kit are detected by the Real-time PCR method by a 5'-nuclease assay with **FAM** labeled probes.

Enclosed controls in the kit:
Internal Control: the PCR mixes contain an **Internal Control IC** probe labeled with **ROX** and with corresponding primers. The **IC** is for the testing of the presence of inhibitors of the PCR which may lead to false negative results. The target gene is not from human source.

Positive DNA Control: this control is added as an extra tube and contains copies of the KRAS gene with mutations. The mix has 5% of mutant DNA-copies of each of 7 mutations of KRAS (for mutation list, see Table 1) and normal human genomic DNA;

Negative Control: as negative control PCR grade water is added.

The PCR mastermixes contain all reagents except **Taq DNA-polymerase** that is supplied in a separate tube and have to be added before the assay.

The KRAS mutation assay provided by the kit includes two parts:

Step 1: Evaluation of unknown DNA Samples by Control PCR (CO-PCR).

First the **Control PCR assay** has to be performed to test if the unknown DNA - extracted from clinical samples - is suitable and well concentrated for the KRAS test to achieve best results. The quality and the quantity of DNA for the KRAS test have to be defined by the Ct of the sample in the **Control PCR.**

Note: A determination of DNA concentration by measurement of the optical density at 260 nm may not provide the correct concentration of DNA and whether the DNA is suitable for the test. The reasons can be a partial degradation and chemical modifications of DNA during the tissue fixation that may inhibit or decrease efficiency of PCR.

This assay is specific for a constant region of the KRAS gene. To test and compare different dilutions of unknown samples the kit are additionally supplied with the Positive DNA Control provided with the kit. For each unknown DNA sample a dilution should be identified that has the Ct value most close to the Ct of the Positive DNA control. The selected dilutions of unknown DNA samples are tested in the Part II of the assay.

Step 2: Allele-specific real time PCR for KRAS mutations (AS-PCR).
After dilutions of the clinical DNA samples are optimized, the "unknown" DNA samples are tested in 7 allele-specific real-time PCR assays using 7 PCR mixes with primers specific to the corresponding mutations of codons 12 and 13 of the KRAS gene (Table 1).

If DNA sample has no mutation (wild type), the Ct in allele-specific real time PCR increases more than 5 cycles in comparison to DNA with mutation. Control PCR reaction should be repeated for all samples. A positive DNA control (supplied) as well as no template control (PCR-grade water) should be included in each PCR run.

The result of allele-specific PCR (AS-PCR) for each mutation is analyzed by comparison of **dCt value of the unknown DNA sample (dCt_{sample})** with **dCt value of Positive DNA control (dCt_m)** (Fig.1). Values for **dCt_{sample}** and **dCt_m** are determined by the equation:

$$dCt_{sample\ or\ m} = Ct_{AS} - Ct_C$$

where (Ct_{AS}) is Ct value in allele-specific reaction, (Ct_C) is Ct value in control reaction;

If the $dCt_{sample} \leq dCt_m$, the DNA sample is positive and has the mutation.

If the $dCt_{sample} > dCt_m$, the DNA sample is negative (no mutation or percentage of mutant KRAS alleles is less than 5%) (See Table 3 for example).

Table 3. Analysis of mutation KRAS-G12V in tumor DNA samples.

Sample*	Ct_{AS}	Ct_C	dCt**	Result
CRC #1	34.32	19.25	15.07	Normal
CRC #2	18.77	16.57	2.20	Mutant
CRC #3	30.35	18.53	11.82	Normal
LC #4	27.29	16.88	10.41	Normal
Positive DNA control	21.27	17.06	4.21	-

**CRC – colorectal cancer; LC – lung cancer; **dCt = $Ct_{AS} - Ct_C$.*

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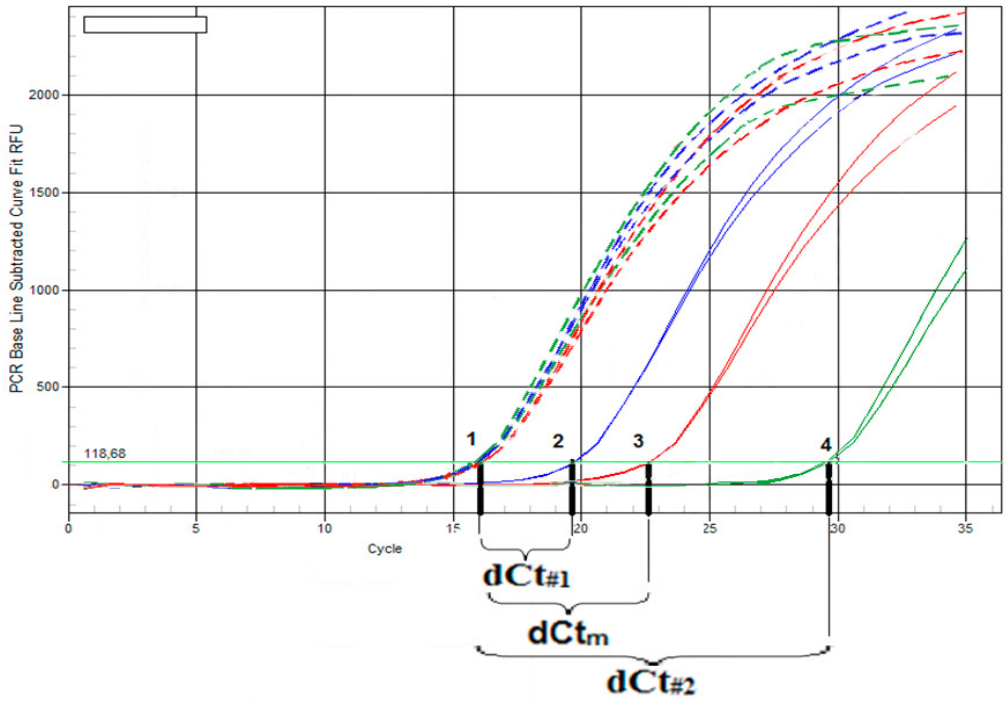

Figure 1. Schematic fluorescence curves for control PCR (dashed lines) and allele-specific PCR (solid lines) of Positive DNA control (red line) and Unknown DNA samples (blue and green lines). **1** - Ct_C for Positive DNA control and Unknown DNA samples, **2** - Ct_{AS} for Unknown DNA sample #1, **3** - Ct_{AS} for Positive DNA control, **4** - Ct_{AS} for Unknown DNA sample #2. DNA sample #1 is positive ($dCt\#1<dCt_m$). DNA sample #2 is negative ($dCt\#2>dCt_m$). DNA samples without mutation could also show Ct_{AS} values more than 35.

7. MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Disposable powder-free gloves and coat;
- Real-Time PCR Device, validated cyclers see page 2
- 8-Well Flat Cap PCR Strips or 96-Well PCR Plates;
- Pipetting tools (0.5 -10 µl, 5 – 50 µl; 20 – 200 µl, 200 – 1000 µl) with sterile filter tips;
- Sterile 1.5 ml microcentrifuge tubes;
- Microcentrifuge (10 000 rpm);
- Vortex;
- Refrigerator +2…+8 °C, Freezer with -15…- 25 °C;
- Heating block for +37…+95 °C;
- Reagents or kit for extracting DNA from FFPE tissue, e.g. RealLine FFPE DNA Extraction Kit (BIORON Diagnostics, REF MED20301) or RealLine FFPE DNA Extraction Mag Kit (BIORON Diagnostics, REF MED32501).

8. SHIPPING AND STORAGE

- RealLine KRAS Detect Kit is shipped with cooling blocks.
- All components of the kit should be stored at (-15 -25) °C.
- Avoid more than 4-5 freeze/thaw cycles.
- Do not expose to light. russo

Attention! *If kit reagents are not frozen on arrival or the package has been damaged during transportation, please contact BIORON Diagnostics GmbH (techsupport@bioron.de).*

9. PRODUCT USE LIMITATIONS

- Kit is for research use only;
- The kits must be used by skilled personnel only;
- Strictly follow the manufacturer's instructions to obtain optimal results;
- Use only the Taq DNA polymerase that is provided with the kit;
- Do not pool reagents from different lots;
- Do not use kit after expiration date.

10. PRECAUTIONS

- All kit components in the working concentrations are non-toxic;
- Follow good laboratory practice when conducting DNA amplification;
- Use a separate room (PCR hood) for each step of analysis, extraction and PCR setup
- Wear a laboratory coat;
- Wear powder-free disposable gloves;
- Use a separate set of pipettes for each step of analysis;
- Use only disposable consumables (filtertips, tubes, etc.);
- The disposal of the used components should be carried out in accordance with the regulations of the respective country.

11. THINGS TO REMEMBER BEFORE PROCEDURE

- Incubate all for the respectively test necessary components for 15 minutes at room temperature to thaw;
- Mix controls and PCR mixes on vortex and spin briefly to collect solution before opening;
- Do not vortex Taq DNA polymerase or any mix containing it;

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12. TEST OF UNKNOWN DNA SAMPLES IN CONTROL PCR (STEP 1)

Note: It is strongly recommended to perform duplicates from all samples and controls. PC (Positive DNA Control) and NC (No template or water control) should be a added to each experiment.

12.1. Preparation of dilutions of DNA samples

To optimize the results the right concentration of DNA is necessary. Test different dilutions for this purpose in this first test.
We recommend from our knowledge for the majority of clinical DNA samples a 5 to 20 -fold dilution is sufficient. Prepare two 1.5ml tubes for each DNA sample. Dilute DNA in PCR-grade water according to Table 4. Mix on vortex and spin the tubes briefly to collect solution at the bottom. You can store diluted DNA for 1 day at +4 °C or up to 12 months at -15…-25 °C.

Table 4. Preparation of DNA dilutions.

Dilution ratio	DNA	PCR-grade water
1:5	30 µl	120 µl
1:20	10 µl	190 µl

12.2. Preparation of PCR mix

- Calculate the amount of samples and mix the Control PCR Mix and Taq-Polymerase:

- Use amount of reagents per reaction as given in Table 5 and scale-up volumes by factor (4N+4) x 1.1, where N = number of analyzed clinical DNA samples.

  Table 5. Reagents per reaction:

Control PCR mix	19.8 µl
Taq DNA polymerase	0.2 µl

- Prepare ready-to-use PCR mix according to calculation as described. First, add Control PCR mix (green cap) into a clean 1.5ml microcentrifuge tube, then add Taq DNA polymerase (tube #9, blue cap) and mix gently by pipetting 8-10 times (Do not vortex!). Place the rest of PCR mix #1 and Taq DNA polymerase at -15…25°C immediately.

- Add **20 µl** of Control PCR mix with Taq DNA polymerase to plate/strip wells.

- Add **5 µl** of each DNA sample (dilutions 1:5 and 1:20), Positive DNA control and PCR-grade water to appropriate wells in duplicate.

- Seal plate/strips with caps or sealing film. Mix gently by tapping with fingers on the side of the plate/strips.

- Centrifuge for 2 min at 2000 rpm to remove bubbles and collect solution at the bottom of the wells.

- Place the plate/strips into block of real-time PCR cycler.

12.3. Programming of the Real-Time PCR Cycler

For basic information about programming of the real-time PCR instrument please read the appropriate Instruction manual. For detailed information about using of the kit with specific real-time PCR cycler please contact BIORON Technical Support (techsupport@bioron.de)

Program the cycler with the temperature profile and fluorescence acquisition according to the Table 5. Use the Ramp Rate 3 ˚C/sec

Table 6. Protocol for KRAS control and KRAS allele-specific PCR.

Cycle number	**Step**		**Temperature**	**Time**	**Optical Data collection**
1	1	Initial activation	95˚C	3 min	No
10	1	Denaturation	95˚C	15 sec	
	2	Annealing	**56˚C**	30 sec	
	3	Extension	72˚C	20 sec	
35	1	Denaturation	95˚C	15 sec	
	2	Annealing and Extension	**56˚C**	60 sec	Yes

Select channels:

- **FAM** (green channel) for detection of the KRAS DNA
- **ROX** (red channel) for detection of the Internal Control DNA.

Reaction volume: 25 µl

Specify the samples and controls location according to prepared plate/strips.

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12.4. Data analysis

Check Ct values of the **Internal Control IC** in **ROX:**

- All samples, PC and NC have to show an amplification signal and CT values have to be determined.
- If a sample does not show a signal, an inhibition of the PCR have occurred. Please repeat the test or in case of the result from one dilution of a sample, discard the result and choose the better working dilution.

Negative Control NC:

- In **ROX** channel an amplification signal should be detected.
- In **FAM** channel no signal or an unspecific background signal after Ct of 33 is expected. Signals with Cts <33 show contaminations. The test has to be repeated.

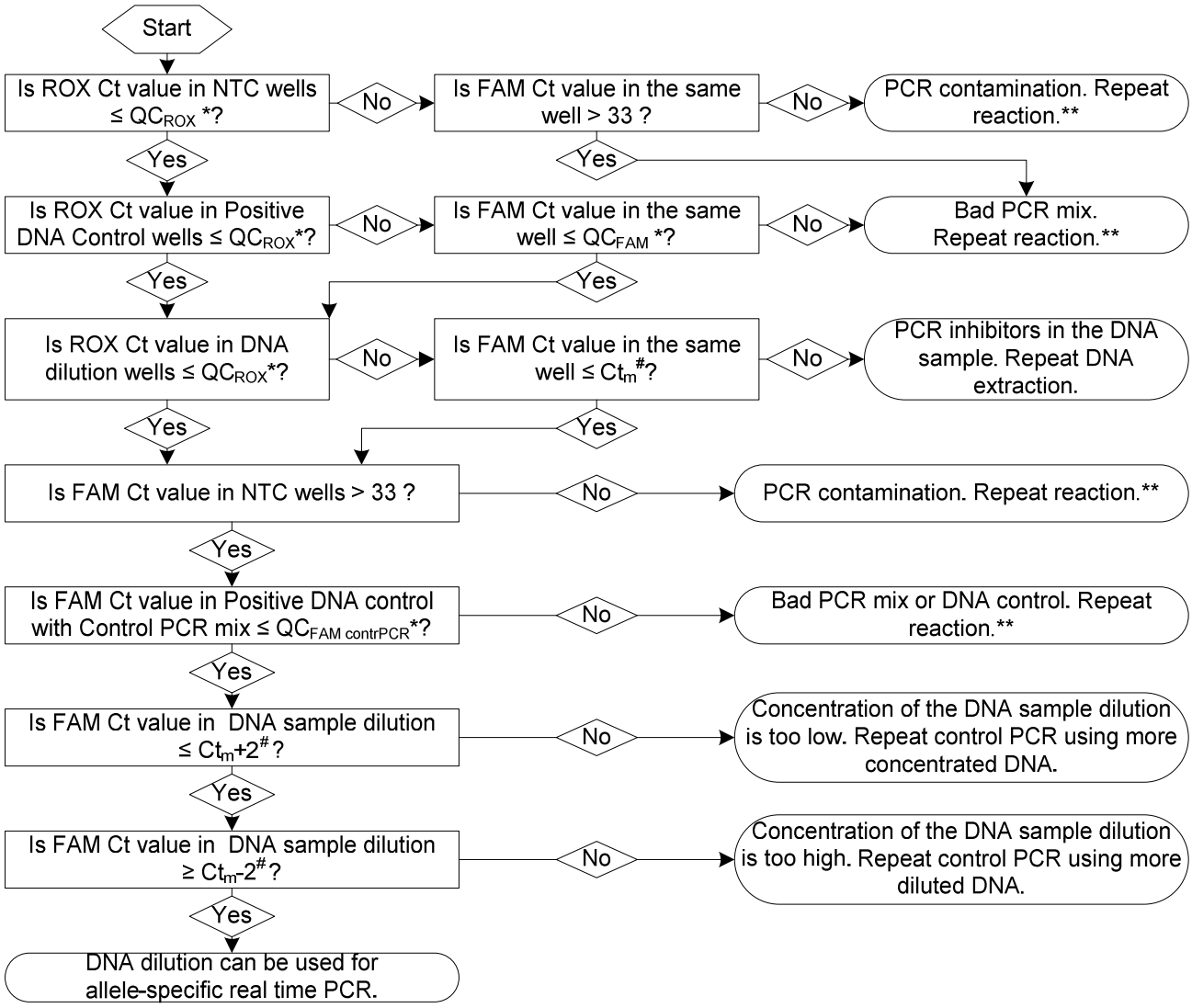
Positive Controls PC:

- In **ROX** channel an amplification signal should be detected.
- In **FAM** channel a clear signal is expected, please notify the QC_{FAM} value at the label of the box and compare the data
  If Ct PC_{FAM} ≤ Ct QC_{FAM}: the result is acceptable, the PCR performance is OK and results can be evaluated.
  If Ct PC_{FAM} > Ct QC_{FAM} indicates that PCR mix or PC DNA is bad.

Sample DNA:

- In **ROX** channel an amplification signal should be detected.
- In **FAM** channel a clear signal is expected:
- **For best results in the Allelic-PCR (Step 2) choose the dilution which is most similar to the Positive Control results.** A dilution of clinical DNA sample is suitable for the KRAS test if in the **FAM** channel Ct of the DNA is different from Ct of the Positive DNA control not more than +/- 2 cycles.

Figure 2. Flow chart for analysis of the control PCR data for the selection of optimal DNA sample dilution.



* Please see QC_{ROX} and $QC_{FAM\ contrPCR}$ lot-specific values in quality control chart supplied with the kit.
** The data must be discarded as data may lead to false results. Repeat PCR for all samples with corresponding and control PCR mix. If Ct value is in the same range again, this PCR mix should be changed for a new one.
# Ct_m – Ct of Positive DNA control.

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13. Test of KRAS mutations by allele-specific real time PCR (Step 2)

Note: It is strongly recommended to perform duplicates from all samples and controls. PC (Positive DNA Control) and NC (No template or water control) should be a added to each experiment.

13.1. Preparation of PCR mix

- Prepare **eight** clean 1.5 ml microcentrifuge tubes. Label it from #1 to #8. Calculate amount of PCR mixes and Taq DNA polymerase.
  Use the amount of reagents per reaction as given in following table and scale-up volumes by factor **(2N+4) x 1.1,** where N - number of analyzed clinical DNA samples. We recommend testing each DNA sample in duplicates.

  Table 7. Reagents per reaction:

PCR mix	19.8 µl
Taq DNA polymerase	0.2 µl

- Up to four unknown DNA samples can be tested in a 96-well plate. In this case add 261.4 µl of each PCR mix #1-8 to corresponding tubes #1-8. Add 2.6 µl of Taq DNA polymerase to tubes #1-8 and gently mix by pipetting 8-10 times (Do not vortex!).

- Add **20 µl** of ready-to use PCR mixes to appropriate wells of 96-well PCR plate or PCR strips. Example of plate setup to test 4 DNA samples is shown (Fig. 4).

- Use optimal dilutions of unknown DNA samples as determined at step 11.4.

- Add **5µl** of DNA sample #1 to all wells of column 1 and 2, DNA Sample #2 to all wells of column 3 and 4, etc. (Fig. 3).

- For NC add 5 µl of PCR-grade water (white cap). For PC add 5 µl add Positive DNA control (red cap).

- Seal the plate/strips with caps or sealing film. Mix gently by tapping with fingers along the side of the plate/strips. Centrifuge for 2 min at 2000 rpm to eliminate bubbles and collect solution at the bottom of the wells.

- Place the plate into the real-time PCR cycler and run PCR as described in step 11.3.

	Sample DNA 1		Sample DNA 2		Sample DNA 3		Sample DNA 4		NC H_2O		PC		PCR-mix
	1	2	3	4	5	6	7	8	9	10	11	12	
A	x	x	x	x	x	x	x	x	x	x	x	x	**Co**
B	x	x	x	x	x	x	x	x	x	x	x	x	**G12C**
C	x	x	x	x	x	x	x	x	x	x	x	x	**G12S**
D	x	x	x	x	x	x	x	x	x	x	x	x	**G12R**
E	x	x	x	x	x	x	x	x	x	x	x	x	**G12V**
F	x	x	x	x	x	x	x	x	x	x	x	x	**G12D**
G	x	x	x	x	x	x	x	x	x	x	x	x	**G12A**
H	x	x	x	x	x	x	x	x	x	x	x	x	**G13D**

Figure 3. Example of a plate setup for KRAS test of 4 unknown DNA Samples, Negative control (H_2O) and Positive control (DNA). Ready-to-use PCR mixes #1-8 (1 control and 7 mutations) are added to rows A-H accordingly. All samples are tested in duplicates. Unknown DNA samples 1-4 are added to wells in columns 1-8; Positive DNA control (DNA C) is added to wells in columns 9 and 10; PCR-grade water is added to wells in columns 11 and 12.

13.2. Data Analysis

Fast Evaluation of the Datas:
Check Ct values of the **Internal Control IC** in **ROX:**

- All samples, PC and NC have to show an amplification signal and CT values have to be determined.
- If a sample does not show a signal, an inhibition of the PCR have occurred. Please repeat the test or in case of a single dilution of a sample, discard the result and choose the better working dilution.

Negative Control NC:

- In **ROX** channel an amplification signal should be detected.
- In **FAM** channel no signal or an unspecific background signal after Ct of 33 is expected. Signals with Cts <33 show contaminations. The test has to be repeated.

Positive Controls PC:

- In **ROX** channel an amplification signal should be detected.
- In **FAM** channel a clear signal is expected, please notify the QC_{FAM} value at the label of the box and compare the data
  If **Ct PC_{FAM} ≤ Ct QC_{FAM}** the result is acceptable, i.e. PCR performance on PC is not bad and results can be evaluated.
  If **Ct PC_{FAM} > Ct QC_{FAM}** this indicates that PCR mix or PC DNA is bad.

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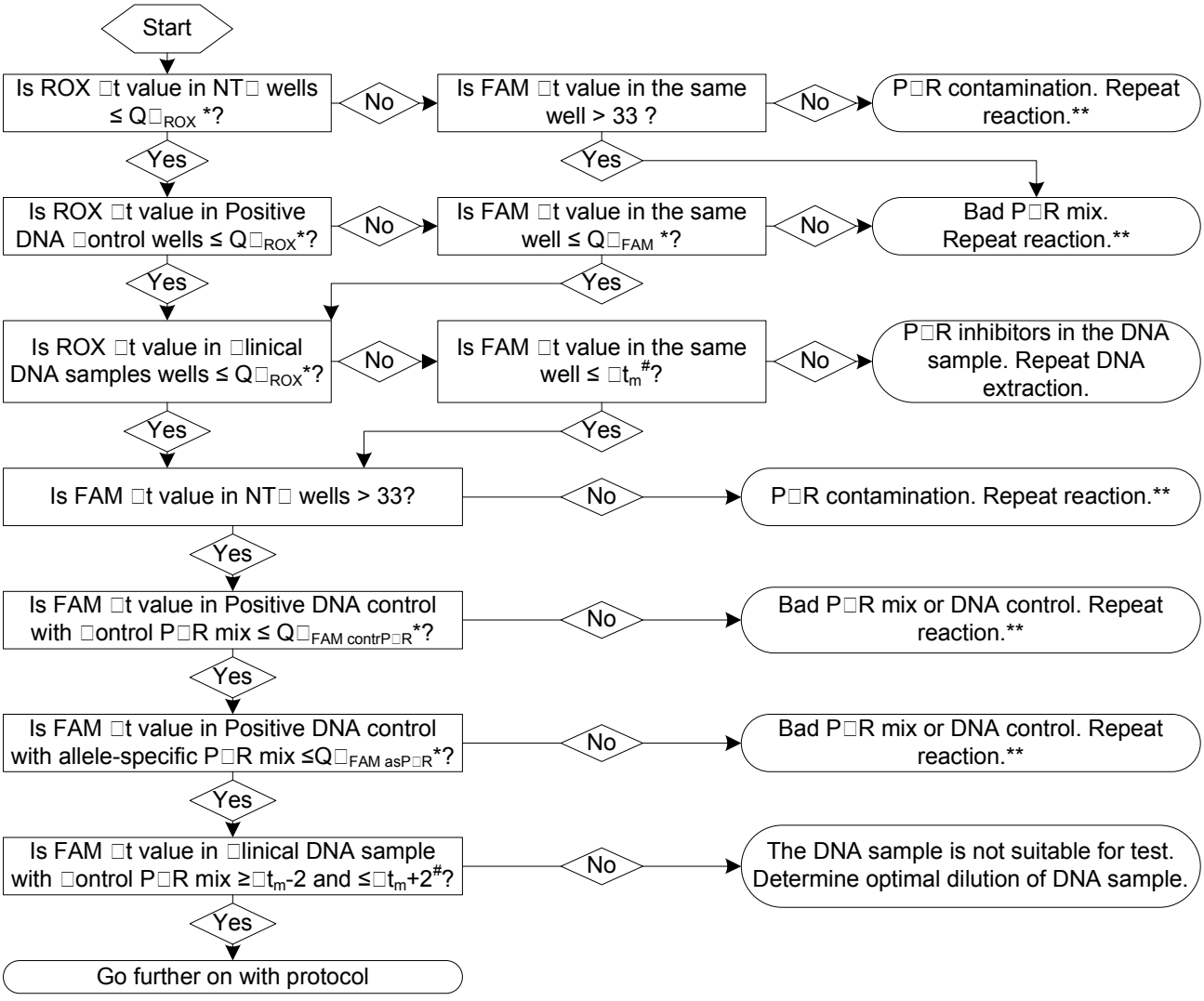
Sample DNA:

- In **ROX** channel an amplification signal should be detected.
- In **FAM** channel: compare each signal to the PC. If the sample has an amplification signal this should maximum differ 4 to the PC. In this case the sample is positive for the mutation!
- Is no signal in the **FAM** channel detectable the sample is negative for the mutation.
- If $\mathbf{Ct_{sample}}$ differs more than 5 cycles from $\mathbf{Ct_{PC}}$ the sample is also negative. The curve is attending to background.

Figure 4. Flow chart for check of the PCR data suitability for the detection of mutation.



** Please see QC_{ROX}, $QC_{FAM\ contrPCR}$ and $QC_{FAM\ asPCR}$ lot-specific values in quality control chart supplied with the kit.*

*** The data must be discarded as data may lead to false results. Repeat PCR for all samples with corresponding and control PCR mix. If Ct value is in the same range again, this PCR mix should be changed for a new one.*

 # Ct_m – Ct of Positive DNA control in reaction with corresponding PCR mix.

Analysis of the PCR data with calculation:

- If Ct value in **FAM** channel for DNA sample cannot be determined (is 0 or N/A) replace it with **"35"**.

- Check standard deviation values for duplicate DNA samples (data in Ct Std. Dev column). If:

  $$\mathbf{Ct_{sample} \leq Ct_m + 3 \text{ and } Ct\ Std.\ Dev > 0.4}$$

  data must be discarded as data may lead to false results. Repeat the PCR!

- For Positive DNA control calculate $\mathbf{dC_m}$ values for each KRAS mutations as following:

  $$\mathbf{dCt_m = Ct_{ASm} - Ct_{Cm}}$$

  $\mathbf{dCt_m}$ is dCt value for Positive DNA control in reaction for a particular mutation,
  $\mathbf{Ct_{ASm}}$ is mean Ct value of a Positive DNA control in allele-specific reaction for a particular mutation,
  $\mathbf{Ct_{Cm}}$ is mean Ct value of a Positive DNA control in control reaction.

- Analogously, for unknown DNA sample calculate $\mathbf{dCt_{sample}}$ values for each KRAS mutations by subtracting mean Ct value for sample in control PCR ($\mathbf{Ct_{C\ sampleN}}$) from mean Ct value for the same sample in allele-specific reaction for a particular KRAS mutation ($\mathbf{Ct_{AS\ sampleN}}$):

  $$\mathbf{dCt_{sample} = Ct_{AS\ sampleN} - Ct_{C\ sampleN}}$$

- Compare $\mathbf{dCt_{sample}}$ value with corresponding $\mathbf{dCt_m}$ for each mutation. (Table 7).

Table 7. Analysis of allel-specific real-time PCR data for KRAS test.

Mutation / PCR mix #	**Positive Control***		**Sample N****
G12C / PCR mix 2	$dCt_{2m}=Ct_{AS2m}-Ct_{Cm}$	←**Compare**→	$dCt_{2\ sampleN}=Ct_{AS2\ sampleN}-Ct_{C\ sampleN}$
G12S / PCR mix 3	$dCt_{3m}=Ct_{AS3m}-Ct_{Cm}$	←**Compare**→	$dCt_{3\ sampleN}=Ct_{AS3\ sampleN}-Ct_{C\ sampleN}$
G12R / PCR mix 4	$dCt_{4m}=Ct_{AS4m}-Ct_{Cm}$	←**Compare**→	$dCt_{4\ sampleN}=Ct_{AS4\ sampleN}-Ct_{C\ sampleN}$
G12V / PCR mix 5	$dCt_{5m}=Ct_{AS5m}-Ct_{Cm}$	←**Compare**→	$dCt_{5\ sampleN}=Ct_{AS5\ sampleN}-Ct_{C\ sampleN}$
G12D / PCR mix 6	$dCt_{6m}=Ct_{AS6m}-Ct_{Cm}$	←**Compare**→	$dCt_{6\ sampleN}=Ct_{AS6\ sampleN}-Ct_{C\ sampleN}$
G12A / PCR mix 7	$dCt_{7m}=Ct_{AS7m}-Ct_{Cm}$	←**Compare**→	$dCt_{7\ sampleN}=Ct_{AS7\ sampleN}-Ct_{C\ sampleN}$
G13D / PCR mix 8	$dCt_{8m}=Ct_{AS8m}-Ct_{Cm}$	←**Compare**→	$dCt_{8\ sampleN}=Ct_{AS8\ sampleN}-Ct_{C\ sampleN}$

* dCt_{Xm} - dCt value of Positive DNA control for mutation that corresponds to PCR mix #X
Ct_{ASXm} – mean Ct value of Positive DNA control in reaction with PCR mix #X.
Ct_{Cm} – mean Ct value of Positive DNA control in reaction with control PCR mix
** N – number of sample
$dCt_{X\ sampleN}$ - dCt value of Sample N for mutation that corresponds to PCR mix #X
$Ct_{ASX\ sampleN}$ – mean Ct value of Sample N in reaction with PCR mix #X
$Ct_{C\ sampleN}$ – mean Ct value of Sample N in reaction with control PCR mix

The DNA sample is positive (has mutation) if the $dCt_{sample} \leq dCt_m$.

The DNA sample is negative (has no mutation or percentage of mutant KRAS alleles is less than 5%) if the $dCt_{sample} > dCt_m$.

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14. REFERENCES

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3. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/000558/WC500029119.pdf
4. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/000741/WC500047710.pdf
5. http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm172905.htm

15. RECOMMENDATIONS FOR DNA ISOLATION FROM FFPE TISSUE BLOCKS

- To extract DNA from FFPE tissue:
- Cut off any excess paraffin with scalpel and cut serial 5 µm sections with a surface area of up to 25-80 mm^2 per section.
- If tissue block has been exposed to air for a long time, discard the first 2-3 sections and use the next 6-8 sections for test.
- The first and the last sections are reserved for pathologist to estimate percentage of tumor cells.
- Place remaining 4-6 sections in a 1.5 ml microcentrifuge tubes for DNA extraction.

We recommend using RealLine FFPE DNA Extraction Kit (BIORON Diagnostics, REF MED20301) or RealLine FFPE DNA Extraction Mag Kit (BIORON Diagnostics, REF MED32501) for DNA isolation from FFPE tissue blocks.

DNA isolated from paraffin blocks may be partially degraded or contain PCR inhibitors. Therefore, efficiency and sensitivity of PCR may vary dramatically for DNA extracted from different paraffin blocks. It is strongly recommended to test DNA performance in control PCR before testing for mutations. For that reason additional amount of control PCR mix is provided in the kit.

Technical Support:

For troubleshooting or technical assistance, please contact the technical support department of BIORON Diagnostics GmbH:

techsupport@bioron.de

RealLine KRAS Detect

RealLine KRAS Detect

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