Devyser KRAS-BRAF

Art. No.: 8-A050

For in vitro Diagnostic Use

Instructions for Use

CE

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1. Introduction to Devyser KRAS-BRAF

Intended use

The Devyser KRAS-BRAF kit is an in vitro diagnostic kit for identification of mutations in the KRAS and BRAF genes.

Devyser KRAS-BRAF is not intended to diagnose cancer. The results from Devyser KRAS-BRAF are intended to be used as an aid to the selection of appropriate drug therapy treatment for patients with colorectal cancer. Clinical decisions for patient treatment should not be made based on the results of Devyser KRAS-BRAF alone.

Included in the kit

The Devyser KRAS-BRAF kit contains ready-to-use reagents for PCR amplification of genetic markers.

Test procedure

DNA extraction: The Devyser KRAS-BRAF kit has been validated using the QIAamp DNA FFPE Tissue Kit (Qiagen, cat#56404) for extraction of DNA from formalin fixed paraffin embedded (FFPE) tissue samples. RNase A treatment during DNA extraction is essential for optimal results.

Amplification: The Devyser KRAS-BRAF kit has been validated using ABI GeneAmp® Systems 9700.

Detection: Applied Biosystems Genetic Analyzers (ABI PRISM® 310, 3100, 3130, 3500, 3730) that support the detection of Devyser Dye-Set DEV-5 or ABI Dye-Set D (DS-30).

Background

Mutations in KRAS and BRAF genes can indicate prognosis and might prevent the therapeutic success of anti-EGFR therapies since tumors carrying mutant forms of the KRAS and BRAF genes are less likely to respond to anti-EGFR therapy.

Testing for KRAS and BRAF mutations can facilitate the identification of tumors that will not respond to anti-EGFR drugs and shortening the time for identifying alternative treatment options.

Principle of the Procedure

The method employed by the Devyser KRAS-BRAF kit uses multiplex allele specific PCR amplification for detection of point mutations in the KRAS and BRAF genes. The PCR products generated are analysed by capillary electrophoresis (CE) and fluorescent detection on a CE instrument.

The kit also includes a pre-analytic amplification control (PAC). It is recommended that the PAC is used to confirm the presence of amplifiable DNA in a clinical sample before performing the KRAS and BRAF mutation analysis.

2. Warnings and Precautions

A.

Devyser KRAS-BRAF has been validated using a total PCR reaction volume of 25 µl. Changing the reaction volume will compromise the kit performance.

B.

Avoid microbial contamination of reagents when removing aliquots from reagent tubes. The use of sterile disposable aerosol barrier pipette tips is recommended.

C.

Do not pool reagents from different lots or from different tubes of the same lot.

D.

Do not use a kit after its expiry date.

E.

Do not use opened or damaged kit reagent tubes.

F.

Workflow in the laboratory should proceed in a unidirectional manner, beginning in the reagent preparation area and moving to the DNA extraction area and then to the amplification area and finally to the detection area. Pre-amplification activities should begin with reagent preparation and proceed to DNA extraction. Reagent preparation activities and DNA extraction activities should be performed in separate areas. Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas. Gloves should be worn in each area and should be changed before leaving that area. Equipment and supplies used for reagent preparation should not be used for DNA extraction activities or for pipetting or processing amplified DNA or other sources of target DNA. Amplification and detection supplies and equipment should remain in the amplification and detection area at all times.

G.

Handling of kit components and samples, their use, storage and disposal should be in accordance with the procedures defined by national biohazard safety guidelines or regulations.

H.

Wear powder free disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

3. Symbols used on Labels

Lot or batch number


Expiry date


Manufacturer

Number of tests

In vitro diagnostic device


Store below temperature shown

4. Required Material

4.1 Included in the Devyser KRAS-BRAF kit (#8-A050)

Configuration

The Devyser KRAS-BRAF kit contains reagents for analysis of maximum 25 samples.

Components

Cap Colour	Tube Colour	Label	Art.Nr.	Kit Content
Orange	Clear	PCR Activator	4-A018	2 x 25 Tests
White	Amber	Devyser KRAS-BRAF Mix	4-A132	1 x 25 Tests
Yellow	Amber	Devyser PAC Mix	4-A141	1 x 25 Tests

4.2 Required but Not Provided

Reagent Preparation

· Consumables for the Thermal Cycler
· Micropipette/dispenser with aerosol barrier tips or displacement tips (500 µl)
· Disposable protective gloves (powder free)

DNA Extraction

· Reagents and equipment according to manufacturer instructions for use
· RNase A (Qiagen art. No.: 19101)
· Micropipette/multipipette with aerosol barrier tips

Amplification

· Thermal Cycler: ABI GeneAmp® PCR System 9700 using 9600 mode. For use of alternative thermal cyclers the following ramping rates must be applied: heating @ 0.8 C/s, cooling @ 1.6 C/s
· Micropipette/dispenser with aerosol barrier tips or displacement tips (5, 20 µl)

Detection

· Applied Biosystems Genetic Analyzer (ABI 310, 3100, 3130, 3500, 3730)
· Performance optimized polymers: POP-4 or POP-7
· Hi-Di Formamide, Genetic Analysis Grade
· 1x Genetic Analyzer Buffer
· Micropipette/multipipette/dispenser with aerosol barrier tips or displacement tips (1,5 uL, 15 uL)

Size Standard:

Devyser Dye Set DEV-5: 560 SIZER ORANGE (Devyser cat.# 8-A402)

ABI Dye Set D: Gene-Scan-500 ROX Size Standard (ABI cat.#401734/#4310366)

4.3 Dye Set Calibration:

ABI 3100, 3130, 3730
Use DEV-5 Dye Set MultiCap kit (Devyser cat# 8-A401) in the “Any5Dye” dye-set or DS-30 Matrix Standard Kit (ABI cat.#4345827) in Dye set D.

ABI 3500
Use DEV-5 Dye Set MultiCap kit (Devyser cat# 8-A401) and generate the DEV-5 dye set or use DS-30 Matrix Standard Kit (ABI cat.#4345827) and generate Dye set D.

ABI 310 Matrix file generation
Use: DEV-5 Dye Set SingleCap kit (Devyser cat# 8-A400). Run with module file “GS STR POP4 (1 mL) G5.md5”

ABI Dye Set D: 6FAM™, HEX, ROX™ (ABI cat# 401546) and NED™ (ABI cat # 402996). Run with module file “GS STR POP4 (1 mL) D.md4”.

5. Storage and Handling Requirements

A.

Store all components below -18°C.

B.

The activated reaction mixes (prepared by addition of Devyser PAC Mix and Devyser KRAS-BRAF Mix to separate PCR Activator tubes) may be stored at +2 to +8°C for at least 7 days and at below –18 C for at least 90 days. Avoid repeated freeze-thawing.

C.

Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

D.

Do not mix reagents from different kit lot numbers.

6. Sample Requirements

Clinical Samples

The Devyser KRAS-BRAF kit is for use with human genomic DNA extracted from formalin fixed paraffin embedded (FFPE) tissue samples. RNase A treatment during DNA extraction is essential for optimal results.

Use three to five sections (10µM) of the FFPE tissue sample for DNA extraction.

Procedure & Storage

According to manufacturer instructions for use.

Controls

It is recommended that suitable controls such as normal DNA and negative control (no DNA) are included in each run.

7. Instructions for Use

Run Sizes

Each Devyser KRAS-BRAF kit (Art # 8-A050) contains reagents for 25 samples.

It is recommended that the activated reaction mix is dispensed into appropriate PCR reaction tubes after preparation. Before dispensing ensure that the activated reaction mix is properly mixed (see section 7.1). Dispense in 20 µl aliquots and store at below – 18 °C.

To avoid contamination always use un-opened tubes. Any reagents left in opened tubes should be discarded.

7.1 Workflow Devyser KRAS-BRAF kit (Art # 8-A050)

The activated reaction mixes should be prepared before preparing the samples, if the complete process is performed in one day. Only if the samples are prepared the day before amplification or earlier, the opposite order is advisable. The activated reaction mixes are prepared by adding the Devyser KRAS-BRAF and Devyser PAC Mixes to separate PCR Activator tubes.

The Devyser KRAS-BRAF kit has been validated using a total PCR reaction volume of 25 µl. Changing the reaction volume will compromise the kit performance.

1. Centrifuge each tube briefly to collect the content. Do not vortex the tubes at this step.

2. Carefully add 500 µl of the Devyser PAC Mix and Devyser KRAS-BRAF Mix to separate PCR Activator tubes.

3. Mix manually by pipetting 300-500 µl several times from the bottom of each tube.

4. Vortex the activated reaction mix tubes and centrifuge briefly to collect the content.

5. Add 20 µl of the activated reaction mixes to separate PCR reaction tubes.

6. Cap the reaction tubes and centrifuge briefly to collect the contents.

The activated reaction mixes are stable at +2-8°C for at least 7 days and at below –18 C for at least 90 days. Avoid repeated freeze-thawing.

7.2 Sample Preparation and PCR Amplification

DNA Extraction

According to manufacturer's instructions for use.

DNA concentration and DNA purity are important factors for successful testing using the Devyser KRAS-BRAF kit. DNA should be free from contaminating RNAs, proteins and salts. Poor quality DNA may result in increased background or amplification failure. Addition of too much or too little DNA to the PCR reaction can cause amplification failure. When using DNA extracted from FFPE tissue samples a pre-evaluation of the DNA quality with the PAC is recommended (see below).

RNAse treatment of all samples during DNA extraction is strongly recommended in order to obtain optimal results.

It is recommended that alternative DNA extraction methods and sample materials are thoroughly evaluated with the Devyser KRAS-BRAF kit prior to the results being used for diagnostic use.

Addition of Sample

Samples should be added in a dedicated area separated from reagent preparation, amplification and detection areas.

1. Add 5 µl of clinical sample to dedicated PCR reaction tubes containing activated reaction mix (from step 7.1)

2. Cap the tubes and centrifuge briefly to collect the content.

Amplification

Turn on the Thermal Cycler at least 30 minutes prior to amplification.

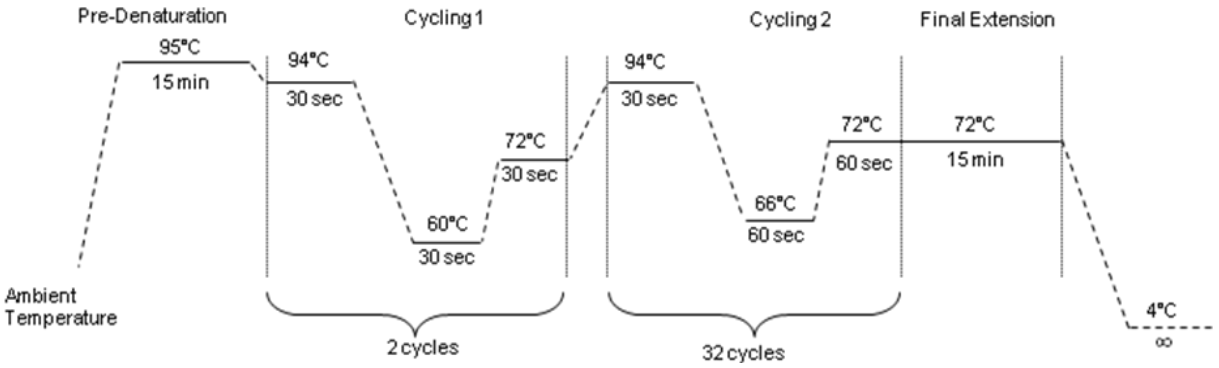
For ABI GeneAmp® System 9700 set “ramp speed” to “9600 mode”.

For use of alternative thermal cyclers the following ramping rates must be applied: heating @ 0.8 ˚C/s, cooling @ 1.6 ˚C/s.

Amplification Area:

Program the Thermal Cycler for amplification according to the following Thermo Profile (consult the User´s Manual for additional information on programming and operation of the thermal cycler):

95°C 15 min
94°C 30 sec - 60°C 30 sec - 72°C 30 - sec for 2 cycles
94°C 30 sec - 66°C 60 sec - 72°C 60 - sec for 32 cycles
72°C 15 min
4°C FOREVER



1. Set reaction volume to 25 µl.

2. Start the amplification (duration approximately 2,5 hrs).

3. Following amplification, remove the tubes containing completed PCR amplification reaction from the thermal cycler and place into a suitable holder. Centrifuge briefly to collect the content. Remove the caps carefully to avoid aerosol contamination. Do not bring amplified material into the pre-amplification areas. Amplified material should be restricted to amplification and detection areas.

7.3 Detection

Sample preparation

Refer to the respective ABI PRISM® Genetic Analyzers User Manual for instructions on maintenance and handling. Prior to running the Devyser KRAS-BRAF kit, the instrument must be spectrally calibrated to support detection of the proper dye-set with the polymer used. See section 4.3 for details.

Sample Preparation for capillary electrophoresis

1. Prepare a loading cocktail by combining and mixing 2 µL of the size standard (e.g. 560 SIZER ORANGE) with 100 µL Hi-Di Formamide (sufficient mix for 6 wells/tubes).

2. Vortex for 15 seconds.

3. Dispense 15 µL of the loading cocktail into the required number of wells of a micro well plate or into individual tubes (ABI310) to be placed on the Genetic Analyzer.

4. Add 1,5 µL of the sample PCR product to the corresponding well/tube containing loading cocktail.

5. Seal the plate/tubes.

Instrument Preparation

Create a sample sheet using the data collection software with the following settings:

- Sample ID
- Dye Set: DEV-5 or D
- Recommended run Module: See below for different polymers and instruments

Run Modules

ABI 310

Run Parameters	POP-4 (ABI 310)
Capillary length	47 cm
Run temperature	60 ˚C
Injection voltage	15 kV
Injection time	5 s
Run voltage	15 kV
Run time	30 min

ABI 3100/3130

Run Parameters	POP-4/POP-7
Capillary length	36 cm
Run temperature	60 ˚C
Injection voltage	1,5 kV
Injection time	20s
Run voltage	15 kV
Run time	1500 s

ABI 3500

Run Parameters	POP-7
Capillary length	50 cm
Run temperature	60 ˚C
Injection voltage	1,6 kV
Injection time	8 s
Run voltage	19,5 kV
Run time	1500 s

The amount of PCR product injected into the capillaries can be adjusted by increasing/decreasing the injection voltage and/or injection time.

8. Results and Analysis

Pre-analytic amplification control (PAC)

A spectrophotometric analysis of the DNA extracted from FFPE tissue may not give reliable results for the assessment of the amount of DNA accessible for PCR amplification. DNA cross linking and fragmentation effects during formalin fixation may limit purification and amplification efficiency. RNA remaining after DNA extraction may also affect spectrophotometric analysis.

Sample analysis using the Devyser PAC Mix helps to determine the degree of DNA degradation and accessibility for PCR amplification.

The Devyser PAC Mix amplifies regions of the KRAS gene to generate fragments of different lengths (figure 1). The product sizes are listed in table 1. The height of the peaks indicate the amount of amplifiable DNA in the sample, the larger the peak height the more amplifiable DNA is present.

It is recommended to use PAC analysis to assess the optimal amount of DNA to be used in the Devyser KRAS-BRAF analysis. The PAC and KRAS-BRAF assays are designed to give similar fragment peak heights for the 97 bp fragment of the PAC analysis and the 115 bp endogenous control fragment of the KRAS-BRAF analysis.

Optimal sensitivity when using the Devyser KRAS-BRAF kit can be expected when the height of the 97 bp PAC peak is between 4000 and 7000 relative fluorescent units (rfu) using an ABI 310, 3100 or 3130 Genetic Analyzer and between 10000 and 25000 rfu using an ABI 3500 Genetic Analyzer. Samples that result in signals exceeding these ranges may lead to false positive results and should therefore be diluted.

The relative peak heights of the different PAC fragments in a sample is an indication of the degree of DNA degradation.

If the 250 bp fragment peak height is significantly lower than the 97 bp and 160 bp fragment peak heights the DNA sample is degraded but the Devyser KRAS-BRAF kit can be used to detect mutations in the KRAS and BRAF genes.

If the 160 bp fragment peak height is significantly lower than the 97 bp fragment peak height the DNA sample is severely degraded. Detection of KRAS or BRAF mutations might still be possible in severely degraded DNA samples if the sample contains a high percentage of mutated tumor material. In this case it is recommended to analyze a new, freshly prepared, DNA sample instead.

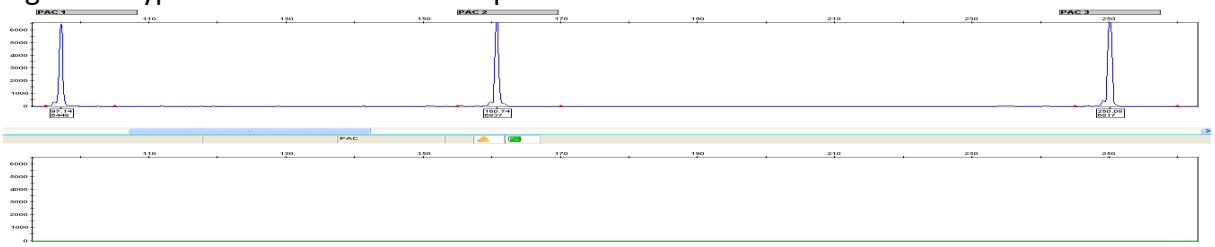
If the 97 bp fragment peak height is very low or absent no KRAS or BRAF mutation can be detected. In this case it is recommended to analyze a new, freshly prepared, DNA sample.

Table 1

Gene	ID	Estimated fragment lengths (bp)*	Window (Colour)
KRAS	PAC 1	97 bp	blue
	PAC 2	160 bp	blue
	PAC 3	250 bp	blue

*Based on observed fragment lengths (basepair, bp) using ABI 3130 and POP7.

Figure 1. Typical results from PAC amplification and detection on an ABI 3130



KRAS and BRAF mutation analysis

The KRAS-BRAF mix amplifies an endogenous control fragment that should be detectable in all samples. The endogenous control fragment should be detected as a peak in the blue window in the size range between 110 bp and 120 bp (figures 2, 3 and 4).

Optimal sensitivity when using the Devyser KRAS-BRAF kit can be expected when the height of the endogenous control peak is between 4000 and 7000 relative fluorescent units (rfu) using an ABI 310, 3100 or 3130 Genetic Analyzer and between 10000 and 25000 rfu using an ABI 3500 Genetic Analyzer. Samples that result in signals exceeding these ranges may lead to false positive results and should therefore be diluted.

KRAS mutations in codons 12 and 13:
A KRAS gene mutation in codons 12 or 13 will be detected in the blue window as a peak in the size range between 135 bp and 165 bp (table 2 and figure 3).

KRAS mutations in codon 61:
A KRAS gene mutation in codon 61 will be detected in the green window as a peak in the size range between 115 bp and 125 bp (see table 2 for details).

BRAF V600E mutation:
A BRAF V600E mutation will be detected in the green window as a peak in the size range between 135 bp and 145 bp (table 2 and figure 4).

Table 2. Summary of PCR fragments detected

Gene	Codon	Mutation		Base Change		Estimated fragment lengths (bp)*	Window (Colour)
Endogenous control fragment						115	blue
KRAS	12	Gly12Ser	G12S	GGT>AGT	c.34G>A	155	blue
		Gly12Arg	G12R	GGT>CGT	c.34G>C	157,5	blue
		Gly12Cys	G12C	GGT>TGT	c.34G>T	163	blue
		Gly12Asp	G12D	GGT>GAT	c.35G>A	149,5	blue
		Gly12Ala	G12A	GGT>GCT	c.35G>C	146	blue
		Gly12Val	G12V	GGT>GTT	c.35G>T	152,5	blue
	13	Gly13Asp	G13D	GGC>GAC	c.38G>A	142,5	blue
		Gly13Ser	G13S	GGC>AGC	c.37G>A	141,5	blue
		Gly13Arg	G13R	GGC>CGC	c.37G>C	144	blue
		Gly13Cys	G13C	GGC>TGC	c.37G>T	140	blue
	61	Gln61His	Q61H	CAA>CAC	c.183A>C	119	green
		Gln61His	Q61H	CAA>CAT	c.183A>T	121	green
BRAF	600	Val600Glu	V600E	GTG>GAG	c.1799T>A	139,5	green

*Based on observed fragment lengths (basepair, bp) using ABI 3130 and POP7.

Example data

Figure 2. Typical results obtained from a sample without mutations

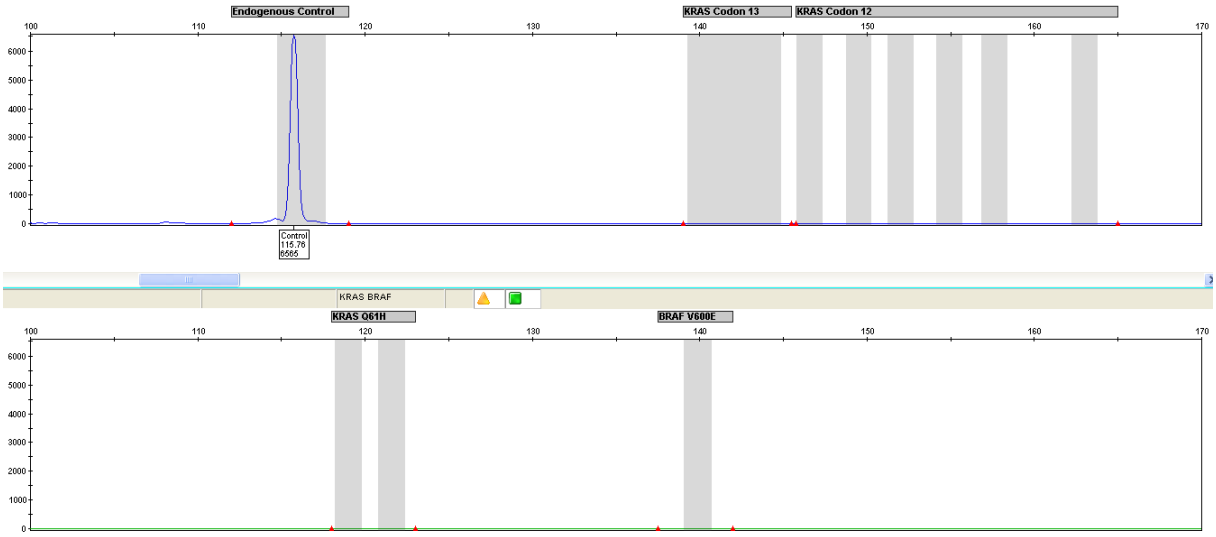


Figure 3. Typical results obtained from a sample containing a KRAS G12D mutation

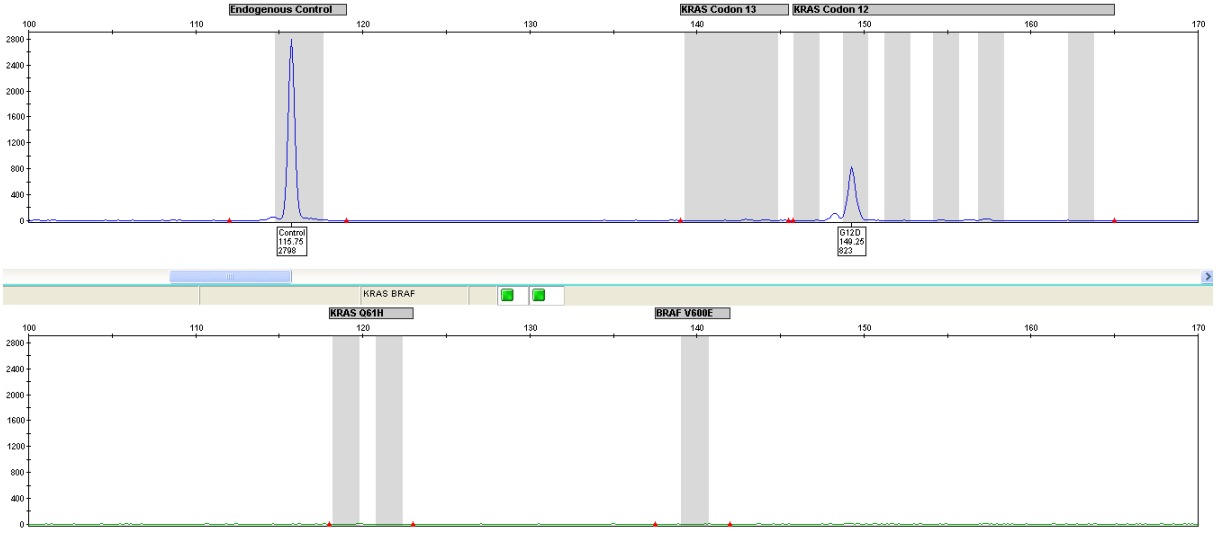
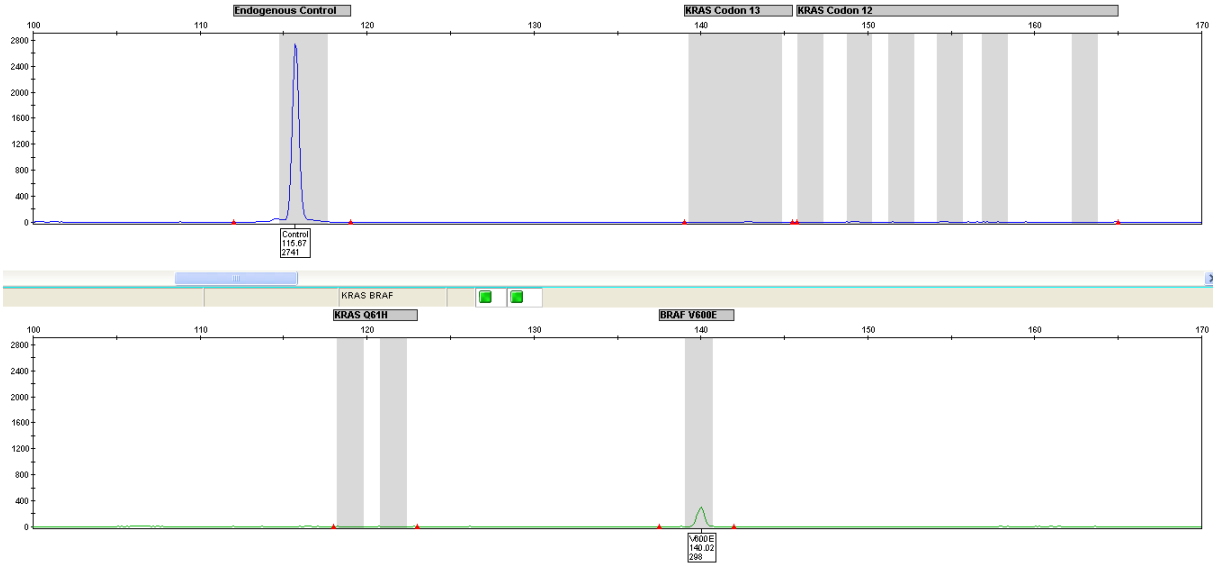


Figure 4. Typical results obtained from a sample containing a BRAF V600E mutation



9. Performance Characteristics

Sensitivity of the Devyser KRAS-BRAF kit

The limit of detection of the Devyser KRAS-BRAF kit has been determined to be <3% mutant sequence in a background of wildtype DNA sequence.

Cross reactivity

The Devyser KRAS-BRAF kit has been shown not to give any false positive mutant peaks when 100 ng wild type cell line DNA (non-formamide treated) is tested.
It has been observed that a G13C positive sample may also give a G13D peak.
It has been observed that a G12V positive sample may also give a G13R peak.
A G12F positive sample may result in a G12V peak.
No other cross reactivity has been observed.

Clinical performance

A total of 28 clinical samples of known KRAS and BRAF genotype (determined by real-time PCR, Sanger sequencing, Pyrosequencing or allele-specific PCR) have been tested with the Devyser KRAS-BRAF kit. Nine samples were provided as DNA, extracted from FFPE materials. Nineteen samples were provided as FFPE material and were extracted by Devyser using the Qiagen FFPE Tissue Kit, including RNase A treatment.

In 24 out of 28 samples the results obtained using the Devyser KRAS-BRAF kit were consistent with the previously identified genotypes. Devyser KRAS-BRAF kit results from four DNA samples were inconsistent with the previously reported results. Further confirmatory testing of the four discrepant samples was performed using pyrosequencing. The confirmatory method produced the same result as Devyser KRAS-BRAF in 3 out of 4 discrepant samples. The fourth discrepant sample was determined as G12V (GTT) using Devyser KRAS-BRAF, G12C (TGT) and G12V (GTT) using allele specific PCR and G12F (TTT) using pyrosequencing.

10. Procedural Limitations

A.

Use of this product should be limited to personnel trained in the techniques of PCR and capillary electrophoresis.

B.

The Devyser KRAS-BRAF kit has been validated using the ABI Thermal Cycler GeneAmp® 9700. It is recommended that alternative thermo-cycler instruments are thoroughly evaluated with the Devyser KRAS-BRAF kit prior to the results being used for diagnostic use.

C.

The Devyser KRAS-BRAF kit has been validated using the QIAamp DNA FFPE Tissue Kit (Qiagen, cat#56404) for extraction of DNA from FFPE tissue samples. RNase A treatment (Qiagen art. No.: 19101) during DNA extraction is essential for optimal results. Performance with other sample materials and DNA extraction kits has not been validated and may result in false negative or false positive results.

D.

Devyser KRAS-BRAF kit should be used only for the detection of specific mutations in the KRAS and BRAF genes according to the instructions for use. The assay has not been validated for diagnosis of cancer. Results obtained with Devyser KRAS-BRAF kit can only be directly applied to the tissue or specific sample material tested.

11. Notice to Purchaser

Results from Devyser KRAS-BRAF kit should be interpreted with consideration of the overall picture obtained from clinical and laboratory findings. Devyser AB will not accept responsibility for any clinical decisions taken.

Purchase of this product does not provide a license to perform PCR under patents owned by any third party.

12. Contact Information

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