

Modaplex BRAF Mutation Analysis Kit

IFU - Instructions for Use



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RUO

For research use only. Not for use in diagnostic procedures.

Intended for use with the Modaplex System.

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Intended Use

The Modaplex BRAF Mutation Analysis Kit is a qualitative and comprehensive PCR-based multiplex assay for the detection of four mutations within the human BRAF oncogene on the Modaplex instrument.

The assay must be used by qualified and trained personnel in a professional laboratory environment only. Results are intended solely for research use and not for diagnostic purposes.

Summary and Explanation

BRAF is a proto-oncogene encoding for a serine/threonine protein kinase B-Raf. It is involved in signal transduction and belongs to the RAS/MAPK pathway regulating cell proliferation, cell differentiation, cellular migration, and apoptosis. When BRAF is mutated, the cellular signaling is altered, leading to several abnormalities including cancer. Point mutations of the BRAF gene were reported in more than 60% of melanomas and at lower rates with 5–15% in colorectal carcinomas¹.

The Modaplex BRAF Mutation Analysis Kit is a fluorescent PCR-based multiplex assay intended for use with the Modaplex platform, which combines qPCR and capillary electrophoresis (CE) in a unique technology. A subset of four BRAF somatic mutations located in codon 600 are amplified using fluorescent-labeled primers that are separated by CE and analyzed through qualitative endpoint detection using Excel validation.

The functionality of the assay is controlled by internal and external controls. Table 1 summarizes the list of BRAF mutation targets.

Table 1: List of assay targets

Target Gene	AA Change ¹	CDs Mutation
BRAF	V600D	c.1799_1800TG>AT
	V600E	c.1799T>A
	V600E-TG/AA	c.1799_1800TG>AA
	V600K	c.1798_1799GT>AA
Control	AA Change ¹	CDs Mutation
B-IC	-	-

¹Amino Acid Residue Change

Principle of the Procedure

The Modaplex platform is a multiplex PCR bench-top system that merges qPCR with a CE-based detection of amplification products in an automated process. This technology - paired with the Modaplex BRAF Mutation Analysis Kit - enables the simultaneous detection and differentiation of multiple targets in FAM and TYE channels through a single reaction.

Oligonucleotide primers are designed to produce PCR products with unique CE mobility. Each individual test includes primers for the four target sites on the BRAF gene and an internal control that targets a wild type portion of the BRAF gene. Each test also includes three size standards (calibrators) used for aligning and assigning CE peaks. At the end of the run, the amplified PCR products are sized using the relative Ct value of the target(s).

Kit format and components

The Modaplex BRAF Mutation Analysis Kit contains reagents that can be used to perform 50 reactions. It includes the following components:

Primer Mix BRAF

This tube contains oligonucleotide primers specific to the four mutations as well as the internal control region on the BRAF gene, as shown in Table 1. Each primer can have either a FAM label or a TYE label, or there can be no label at all.

PCR Buffer 2

This solution is optimized to promote enzyme activity for the PCR in the Modaplex BRAF Mutation Analysis Kit.

Modaplex Polymerase B

The Modaplex BRAF Mutation Analysis Kit contains a Taq polymerase.

Modaplex Enhancer 50

This solution enhances the PCR reaction and is thus essential for the process.

Modaplex Calibrator 2

A liquid concentrate containing DNA template for calibration controls.

10X Injection Buffer

A liquid used by diluting 1:10 to fill those wells in the PCR plate that are not being used for samples.

Control concept

The BRAF Mutation Analysis Kit comes with a comprehensive control concept. It comprises internal and external controls to evaluate the functionality of the PCR reaction and identify potential contamination. The control concept is described below:

Template-independent PCR controls: Calibrators

The Modaplex BRAF Mutation Analysis Kit has been designed to use Modaplex Calibrator 2. In both, the FAM and the TYE channel, three calibrators of different sizes are amplified. They represent a template-independent PCR control and internal length standard.

Table 2: Calibrator lengths in FAM and TYE channels

	Calibrator length (bp) FAM channel	Calibrator length (bp) TYE channel
CAL 1	110	113
CAL 2	249	251
CAL 3	306	309

The Modaplex Calibrator 2 must be added to all sample, negative control and positive control wells.

Internal Control (B-IC)

The Modaplex BRAF Mutation assay contains the internal control, which serves as a template-dependent PCR control in sample wells.

External Control 1: Negative Control (NTC)

The user needs to set up an NTC (no-template control) for each run to assess the potential contamination while setting up the assay. The NTC consists of Nuclease Free Water (H₂O), which is to be used as a template for the NTC well.

Note: The user needs to set up an NTC for each Modaplex run.

External Control 2: Positive Control (PC)

The Positive Control BRAF Mutation contains an artificial template representing each of the four BRAF mutations and the internal control. The positive control results are assessed to ensure that the kit performs within the stated acceptance criteria. All targets must be detectable within acceptable ranges to confirm the proper functioning of the Primer Mix.

Note: The user needs to set up a Positive Control for each Modaplex run.

Platform and software

Modaplex Instrument

The Modaplex BRAF Mutation Analysis Kit is designed to be used with the Modaplex instrument (software version 1.0.23 or higher). This platform is a fully automated bench-top system for molecular diagnostic applications. It combines qPCR with capillary electrophoresis (CE) in an automated process and enables the detection, differentiation, and quantification of up to 50 DNA and RNA targets in a single well and run. Therefore, it enables the individual combination of tests for fragment analysis, mutational analysis, gene expression, copy-number variation, etc.

Excel

It is strongly recommended that the Modaplex BRAF Mutation Analysis Kit is analyzed using the Excel validation sheet.

Materials Provided

Kit contents

Table 3 summarizes the contents of the Modaplex BRAF Mutation Analysis Kit and indicates the required storage conditions.

Table 3: Content of the BRAF Mutation Analysis Kit

Component	Ingredients	Vials	Vol./Vial (µL)	Cap Color	Storage
Primer Mix BRAF	a) FAM or TYE-labeled and unlabeled primer for BRAF mutation b) Control gene primer c) Tris-EDTA buffer	1	100	Red	-15 °C to -25 °C
PCR Buffer 2	a) Deoxynucleotide tri-phosphates b) Buffer	1	625	Black	-15 °C to -25 °C
Modaplex Enhancer 50	a) Magnesium chloride b) Tris-EDTA buffer	1	100	Natural	-15 °C to -25 °C
Modaplex Polymerase B	a) Taq polymerase b) Glycerol	1	25	Orange	-15 °C to -25 °C
Positive Control BRAF	a) Control template b) Tris-EDTA buffer	1	100	White	-15 °C to -25 °C
Nuclease-Free Water	a) Nuclease-free water	1	1.500	Light blue	-15 °C to -25 °C
Modaplex Calibrator 2	a) DNA template for Calibration Controls	2	25	Yellow	-15 °C to -25 °C
10X Injection Buffer	a) Buffer	1	1.500	Purple	-15 °C to -25 °C

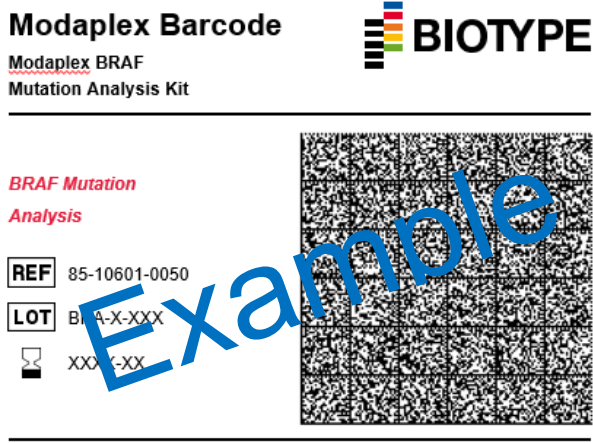
Packaging inserts

The Modaplex BRAF Mutation Analysis Kit comes with two packaging inserts - the Modaplex Barcodes and the IFU Download Instructions. Each kit must be used with the supplied packaging inserts. This is to guarantee that the test is set up according to the latest instructions for use (IFU), and that the correct Modaplex assay definitions are used for BRAF Mutation analysis. Both packaging inserts are described in the following subsections.

Modaplex Barcode

The Modaplex barcode is provided for the detection and quality assessment of both sample and control wells. Prior to the first use of a new lot of the BRAF Mutation Analysis Kit, the barcode must be scanned. On scanning the barcode, BRAF-related assay specifications such as assay name, assay article number, expiration date, lot number, list of targets, and internal controls are automatically transferred to the Modaplex instrument.

Figure 1: Example of Modaplex Barcode



The **Modaplex Barcode** contains all BRAF Mutation Kit-related information required for the detection of BRAF mutations using the sample material.

To ensure full traceability of kit lot numbers and expiration dates, the assay-specific Modaplex barcode needs to be scanned prior to the first use of a Modaplex BRAF Mutation Analysis Kit. With each new lot of the assay, the newly provided barcode needs to be scanned, resulting in a new assay definition on the Modaplex instrument, including the new lot number and expiration date.

Modaplex IFU Download

The BRAF Mutation IFU is not physically a part of the Modaplex BRAF Mutation Analysis Kit. However, to guarantee that the kit is used according to the dedicated IFU, the BRAF Mutation assay comes with a packaging insert labeled "Modaplex IFU Download." It provides guidance on how to download the latest IFU version. This document contains the following information:

- The latest version of the IFU that must be used with the current kit
- Guidance to the access-restricted download area
- Access (IFU) code to enable the download of the IFU

For successful download, please refer to the following short instructions:

1. **Open** Biotype GMBH's webpage using the link provided on the Modaplex IFU download packaging insert:
www.biotype-innovation.com/product/knb/braf/
2. **Click** on the respective IFU link in the BRAF Mutation download section.
3. **Enter** the IFU Code in the respective field.
The IFU Code is given on the packaging insert "Modaplex IFU Download".

Note: If your laboratory environment does not give access to the internet, please contact Biotype GmbH's support at support@biotype.de and request the dedicated IFU.

Materials required (not included in the kit)

In addition to the kit contents, the following reagents, consumables, and equipment are required but not provided with the Modaplex BRAF Mutation Analysis Kit.

Reagents and Consumables

- DNA extraction kit and consumables (QIAGEN Cat# 937236 and Cat# 56404)
- DNA quantification kit and consumables (ThermoFisher Cat# Q32866, Q32850 or Q32851, Q32856)
- Sterile filtered nuclease-free pipette tips (several suppliers)
- Sterile microcentrifuge tubes (several suppliers)
- TE Buffer, pH 8.0 (ThermoFisher Cat#: 10006044)
- PCR Microplates 96 (Biotype)
- Mineral Oil (Sigma-Aldrich)
- Aluminum sealing film (Biotype)
- Modaplex Cartridge CE 48 (Biotype)
- Modaplex Buffer (Biotype)
- Modaplex Decon (Biotype)
- Modaplex Wash (Biotype)
- Pop -7/ Modaplex CE Gel (Biotype)
- Modaplex CE Plates (Biotype)

Instruments and Software

- Modaplex System (Biotype)

Equipment

- Micropipettes (several suppliers)
- Bench-top centrifuge with rotor for 2 mL reaction tubes (several suppliers)
- Bench-top centrifuge with plate adaptor (several suppliers)
- Bench-top vortex (several suppliers)

Warnings and Precautions

For research use only. Not for diagnostic use. Intended for use with the Modaplex instrument

Safety Information

General Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

For more information about the Modaplex BRAF Mutation Analysis Kit components, please consult the corresponding safety data sheets (SDS), which are available on request. For the safety information of any component not provided with the kit, please contact the respective supplier.

Modaplex Safety Information



Attention to the high voltage required for the CE separation. The failure to not fill all wells is a general safety threat that may cause damage to the Modaplex instrument.

General Precautions

The user should pay close attention to the following points:

- Follow good laboratory practice guidelines.
- Only skilled laboratory technicians who are properly trained to perform reactions using PCR technology should be allowed to use this product.
- Clean and disinfect all surfaces according to the laboratory's standard operating procedure (SOP) guidelines.
- Use DNase-, RNase-, and DNA-free pipette tips with filters. Ensure that the pipettes have been calibrated according to the manufacturer's instructions.
- Change pipette tips after each pipetting step to avoid sample mixing and cross-contamination.
- Do not reuse disposables.
- Open and close the reagent containers carefully.
- Follow the instructions for reagent storage and handling.
- Ensure that the reagents are not exposed to light during storage.
- Do not use reagents beyond their expiration dates.
- Any wells in the PCR plate that are not being used for testing a sample must be filled with 25µL of 1x injection buffer, included in the Calibrator 2 kit, and overlaid with Mineral Oil.
- Do not substitute the reagents with comparable reagents from other manufacturers.
- Do not substitute the equipment listed in this document.
- Follow the instructions in the Modaplex System User Manual for the proper operation of the Modaplex System.
- Safety Data Sheets (SDS) are available upon request from Biotype GmbH.

Note: Use extreme caution to prevent the contamination of the PCRs with control material. We recommend using separate, dedicated pipettes for setting up the reaction mixes and adding the DNA template. To avoid potential (cross-)contamination, separate the different procedural steps into at least two distinct working areas - one clean area for the preparation of the PCR master mixes and a second area for the addition of the DNA template samples to the PCR reactions and the operation of the Modaplex instrument.

Note: The reagents are validated for manual setup. Using an automated method may reduce the number of possible reactions due to the reagent required to fill "dead volumes" on these instruments.

Note: All reagents in the Modaplex BRAF Mutation Analysis Kit are formulated specifically for use in the stated test. All the reagents supplied in the kit are intended to be used solely with the other reagents in the same Modaplex BRAF Mutation Analysis Kit. To maintain optimal performance, the reagents in the kit must not be substituted.

Note: Use only the Taq DNA polymerase (Modaplex Polymerase B) provided in the kit. Do not substitute with Taq DNA polymerase from other kits of the same or any other type, or with Taq DNA polymerase from any other supplier.

Reagent Storage and Handling

The Modaplex BRAF Mutation Analysis Kit is shipped on dry ice. Upon receipt, the assay should be stored at $-15\text{ }^{\circ}\text{C}$ to $-25\text{ }^{\circ}\text{C}$ in a constant-temperature freezer and protected from light. The fluorescent-labeled molecules must be protected from light to avoid photobleaching. If stored under the recommended storage conditions in the original packaging, the kit will remain stable until the expiration date stated on the label.

- Repeated thawing and freezing (>3 cycles) should be avoided.
- Do not use expired or incorrectly stored components.
- All kit components are optimally diluted and no further treatment is necessary.

Note: Upon receipt, please check the Modaplex BRAF Mutation Analysis Kit and contact Biotype GmbH's technical service if any of the following problems are observed:

- A component is not properly frozen.
- The kit labels are damaged.
- The outside box has been opened.
- The reagents are missing.
- The packaging inserts (Modaplex Barcodes and IFU Download) are missing

Procedure

The Modaplex BRAF Mutation Analysis Kit has been verified using artificial sample material spiked into the genomic DNA background for each target. Horizon Discovery reference standards derived from human cell lines have been also included in the verification studies. In addition, pre-characterized DNA samples (mutations) extracted from formalin-fixed paraffin-embedded (FFPE) colorectal tissue have been tested for use with the Modaplex BRAF Mutation Analysis Kit.

DNA purification

It is recommended that DNA purified with QIAamp DNA FFPE Tissue Kit (QIAGEN, Cat#: 56404) is used. DNA purification should be carried out from macro-dissected FFPE tissue according to the supplier's instructions.

Note: The Modaplex BRAF Mutation Analysis Kit is designed for use with short PCR products; the assay will not work with heavily fragmented DNA.

Note: After the DNA isolation procedure and prior to storage, the DNA concentration must be promptly measured.

DNA quantification

The DNA should be quantified by fluorometric quantitation using the Qubit™ Fluorometer (version 2 or higher). For low FFPE tissue input (e.g. tissue biopsies), use the Qubit™ dsDNA HS Assay (Cat#Q32851) according to the manufacturer's protocol. Otherwise, the usage of the Qubit™ ds DNA BR Assay (Cat#Q32850) is recommended.

Set up the Modaplex BRAF Mutation Analysis Kit using 10ng (2ng/μl). Using a DNA amount below 10ng will result in low PCR yields and the signal might fall below the target-specific detection limits.

Protocol: Preparing the Modaplex run for BRAF Mutation Analysis

This protocol is intended for the preparation of the Modaplex instrument prior to setting up the Modaplex BRAF Mutation Analysis Kit run.

1. Determine the number of PCR reactions.

Before setting up the Modaplex instrument run, the number of PCR reactions should be determined. The following controls should be included in the calculation:

- One negative control (NTC)
- One positive control (PC)

2. Determine which wells are to be filled with the 1x Injection Buffer.

This number is based on the number of capillaries in the cartridge minus the total number of PCR reactions to be performed.

3. Add the BRAF mutation assay definitions to the Modaplex instrument.

Scan the two Modaplex barcodes provided with the Modaplex BRAF Mutation Analysis Kit. The assay definitions are automatically added to the Modaplex System. Refer to the Modaplex System User Manual for further instructions.

Note: The Modaplex barcodes has to be scanned every time a new lot of the Modaplex BRAF Mutation Analysis Kit is used.

4. Confirm the Modaplex settings for the BRAF Mutation Modaplex run.

Before setting up the Modaplex BRAF Mutation Analysis Kit, the following conditions regarding the consumables should be satisfied for the planned Modaplex run:

- Sufficient number of remaining runs in the Modaplex cartridge
- The quantity of the consumables must be adequate.

Note: To replace the Modaplex System cartridge or the consumables, please refer to the Modaplex System User Manual for further instructions.

5. Create a run definition and a plate map on the Modaplex instrument.

For overall evaluation and data assignment, the Excel Meta-analysis Worksheet requires the following form of **sample-naming**:

- Positive control: assay B-PC
- Negative control: assay B-NTC
- Sample Well: sample name

Note: The sample name can be any unique name, number, or code that can identify a sample.

Note: Sample names must be unique. If there are duplicates on a plate, they must have different names. Avoid special symbols such as double quotes ("), brackets (< or >), and ampersands (&).

Note: Remove all quality-control ticks in the plate setup window as no absolute quantification is taking place.



Figure 2: Plate setup window of the Modaplex software. Left: Quality control fields with ticks; Right: Quality control fields without ticks

Protocol: Setting up the BRAF Mutation Modaplex run

This protocol is to be used for the preparation of reagents in the Modaplex BRAF Mutation Analysis Kit and the PCR plate for the Modaplex run.

1. **Remove and thaw** the following components from the Modaplex BRAF Mutation Analysis Kit:

- PCR Buffer 2 (black cap)
- Modaplex Enhancer 50 (natural cap)
- Primer Mix BRAF (red cap)
- Nuclease-Free Water (light-blue cap)
- Positive Control BRAF (white cap)
- Modaplex Polymerase B (orange cap)
- Modaplex Calibrator 2 (yellow cap)
- 10X Injection Buffer (purple cap)

Note: The Modaplex Polymerase B is temperature-sensitive. Keep the enzyme at $-15\text{ }^{\circ}\text{C}$ to $-25\text{ }^{\circ}\text{C}$ at all times.

2. **Homogenize** the thawed reagents by inverting the tubes, pipetting, or gently vortexing. Thereafter, briefly centrifuge the reagents.

3. **Preparing the BRAF Mutation master mix.**

Prepare the PCR reagent Master mix (MMx) in an appropriately sized microcentrifuge tube for the total number of samples to be tested in a dedicated clean area. Use Table 4 to determine the volume of the reagents.

Table 4: BRAF Mutation Master Mix (MMx): The volumes of the reagents needed for the Master Mix

Component	Volume of Reagents Calculated According to the # of tests					
	# 1	# 2	# 3	# 4	# 5	# 10
PCR Buffer 2	12.5	25	37.5	50	62.5	125
Primer Mix BRAF	2.0	4.0	6.0	8.0	10	20
Nuclease-Free Water	2.97	5.94	8.91	11.88	14.85	29.7
Modaplex Enhancer 50	1.13	2.26	3.39	4.52	5.65	11.3
Modaplex Polymerase B	0.4	0.8	1.2	1.6	2.0	4.0
Modaplex Calibrator 2	1.0	2.0	3.0	4.0	5.0	10
Total Volume of Master Mix (MMx)	20.0 μL	40.0 μL	60.0 μL	80.0 μL	100.0 μL	200.0 μL

Note: As a rule of thumb, if you are testing fewer than 10 samples, use enough Master Mix for one extra sample. If you are testing 10 or more samples, use an excess reagent Master Mix volume of +10%.

5. **Mix gently** by inverting the tube or by pipetting. Spin down briefly in a bench-top microcentrifuge.

6. **Aliquot 20 μL** of the BRAF Master Mix to the designated wells in the PCR plate.

7. **Add 5 μL** of each of the following:

- Extracted DNA (template, optimum 10 ng) to the corresponding sample well(s)
- Nuclease Free Water to the NTC well
- Positive Control BRAF to the positive control well

Note: Thus, the total volume of the PCR reaction is 25 μL in each well. Refer to Figure 3 for an example of the sample plate layout.

8. **Add 25 μ L of 1x Injection Buffer (1x IB)** to the remaining empty wells that are not being used for a sample well, NTC or PC.
 - Prepare a 1:10 dilution of the 10x Injection Buffer with water.
 - Add 25 μ L of the 1x Injection Buffer to each of the empty wells. Please refer to the plate map setup in the Modaplex System IFU for further explanation.



Attention to the high voltage required for the CE separation. The failure to not fill all wells is a general safety threat and may damage the Modaplex instrument.

Figure 3: Example of a sample plate layout for 30 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 01 MMx	Sample 09 MMx	Sample 17 MMx	Sample 25 MMx	Empty 1x IB	Empty 1x IB						
B	Sample 02 MMx	Sample 10 MMx	Sample 18 MMx	Sample 26 MMx	Empty 1x IB	Empty 1x IB						
C	Sample 03 MMx	Sample 11 MMx	Sample 19 MMx	Sample 27 MMx	Empty 1x IB	Empty 1x IB						
D	Sample 04 MMx	Sample 12 MMx	Sample 20 MMx	Sample 28 MMx	Empty 1x IB	Empty 1x IB						
E	Sample 05 MMx	Sample 4 MMx	Sample 21 MMx	Sample 29 MMx	Empty 1x IB	Empty 1x IB						
F	Sample 06 MMx	Sample 14 MMx	Sample 22 MMx	Sample 30 MMx	Empty 1x IB	Empty 1x IB						
G	Sample 07 MMx	Sample 15 MMx	Sample 23 MMx	B-PC MMx	Empty 1x IB	Empty 1x IB						
H	Sample 08 MMx	Sample 16 MMx	Sample 24 MMx	B-NTC MMx	Empty 1x IB	Empty 1x IB						

9. **Seal** the PCR plate with aluminum sealing film. Gently vortex and spin the PCR plate in a table-top centrifuge.
10. **Remove** the seal and **overlay** all 48 wells on the PCR plate with **one drop of Mineral Oil**. Ensure that each well is fully covered by oil.
11. **Seal** the PCR plate again with aluminum sealing film. Spin the PCR plate and CE plate in a table-top centrifuge.

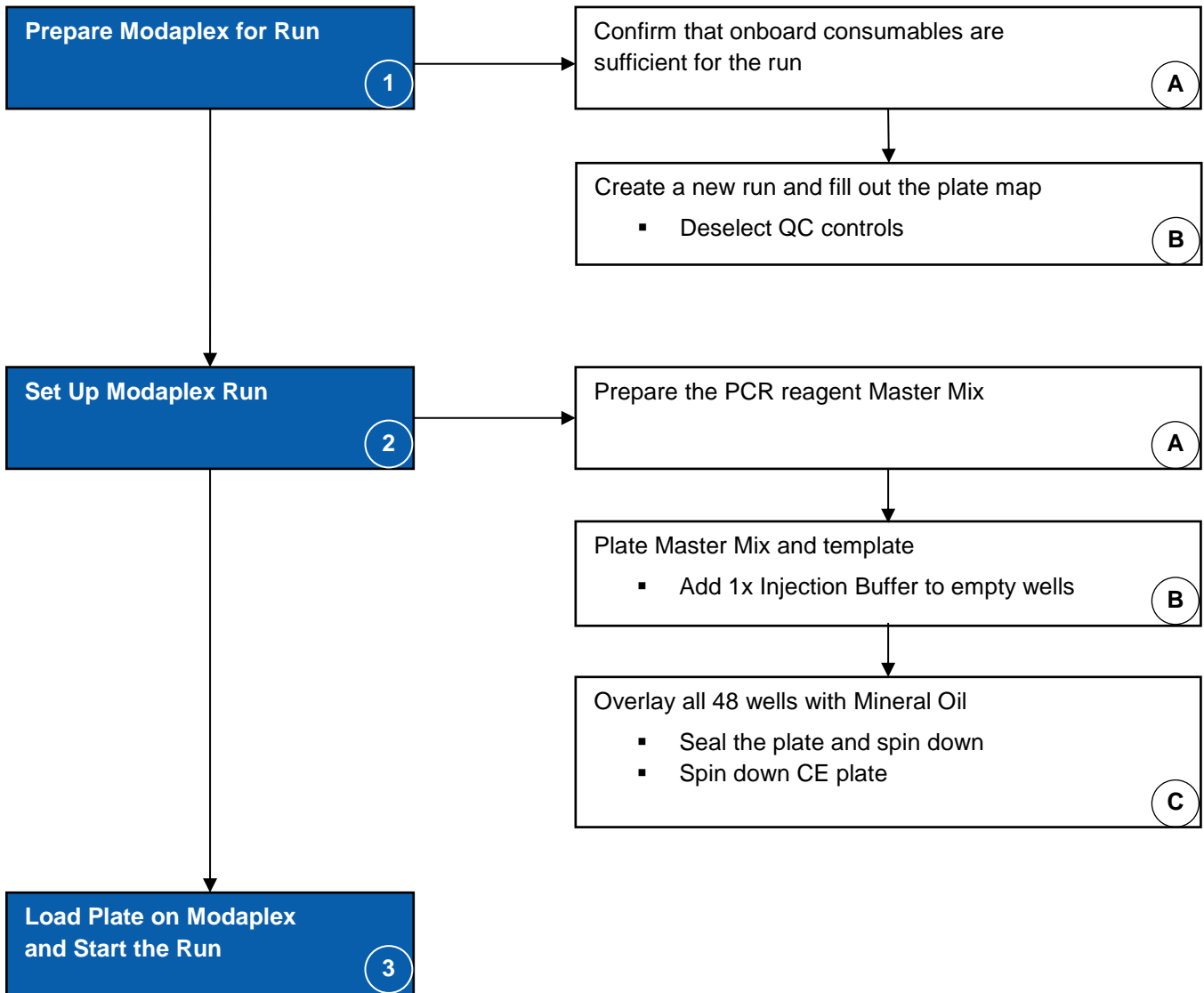


The plate cover seals must be **removed** from the PCR plate and the CE plate before being placed on the Modaplex instrument.

12. **Run the PCR plate** in the Modaplex instrument.
4. **End of Run**
At the end of the run, **seal** the PCR and CE plates with aluminum sealing film before disposing them. Decontaminate the hold-down plate.

Note: Refer to the Modaplex System IFU for further instructions.

Procedure - Flow Chart



Interpretation of the Results

After the Modaplex run is completed, the BRAF data must be analyzed using the following procedure:

1. Data transfer to the Excel Meta-analysis Worksheet
2. Run control analysis
3. Sample analysis

Data transfer to the Excel Meta-analysis Worksheet

The following section describes how the BRAF data is transferred from the Modaplex system to the Excel Meta-analysis Worksheet.

1. **Load** the appropriate completed run on the Modaplex User Application (UA).
2. **Open** the Excel Meta-analysis Worksheet template.
3. **Select** the Run Setup menu.
4. **Select** the Results tab.
5. **Select** the View button on the left side of the screen. The Data Viewer (DA) will open.
6. **Select** Reports on the file bar.
7. **Deactivate** QC Flags.
8. **Click** File.
9. **Select** Export to Clipboard. This loads the Modaplex data into the open Excel Meta-analysis Worksheet.
10. **Pass** in Meta-analysis worksheet in uCt tab into cell A1.
8. **Select** the Result tab in the Excel Meta-analysis Worksheet to review results. The BRAF Mutation Summary analyzes the run controls and samples in detail.

Run Control Analysis

The Excel Meta-analysis Worksheet analyzes the run controls and reports “Pass” or “Failed” for both negative control and positive control. For these wells, targets are evaluated independently as described below, and each target is assigned Pass or Failed. Further analysis may be required. Follow the guidelines below.

- **Negative control:** For the negative control targets to be reported as B-NTC Pass, no amplification of the targets or B-IC is detected, or the amplification level is below the Ct value cutoffs embedded in the Excel Meta-analysis Worksheet. If a negative control target is reported as B-NTC Failed, B-IC (contamination) is detected or the amplification level is below the Ct value cutoffs.
- **Positive control:** For the positive control targets to be reported as B-PC Pass, amplification of at least 50% of all 4 targets is detected.
- **QC Flags:** QC flags are reported by the data viewer. These are not used by the Excel Meta-analysis Worksheet. The user should review any QC messages in the data viewer for validity. See Table 5 below for information on these messages.

Note: When reviewing wells in the data viewer, always review the six calibrator targets to ensure that the well is properly aligned.

Table 5: Possible QC Flags

Message	Explanation	Potential cause and resolution
(A). Low CE quality	Capillary electrophoresis data showed poor quality or the calculated score for the well was higher than 15	Errors during CE electrophoresis are possible. Review well for misalignment or errors. Due to the complexity of this assay, high scores are common and often do not indicate a CE quality issue. If necessary, discard the well or rerun the sample.
(C). Low amplification curve quality	A target was amplified, but the quality of the amplification curve was inadequate for estimating the result for a target	PCR amplification or data analysis error. Review the target for errors. If necessary, discard the well or rerun the sample.

Note: Other QC flags are possible. See Modaplex System User Manual for more information.

Sample Analysis in the BRAF Mutation Summary

The Excel Meta-analysis Worksheet analyzes the samples and reports Dilute, Insufficient Sample Input, Sample Input Sufficient, Mutation Detected, or Mutation Not Detected for every well excluding empty wells. Follow the guidelines below.

Note: All wells are reported excluding the empty wells. If another assay is run on the same PCR plate as the Modaplex BRAF Mutation Analysis Kit (RUO), the results will still be reported for the Modaplex BRAF Mutation Analysis Kit for those wells. The user should disregard these results.

Note: Please do not use the filter function in the Excel Meta-analysis Worksheet.

B-IC (Internal Control): For the sample to be analyzed, first the B-IC must pass the Ct value cut-offs embedded in the Excel Meta-analysis Worksheet (See Table 6). If the Ct value for the B-IC is below the lower cut-off value, the Excel Meta-analysis Worksheet will state Dilute (if BRAF) for all targets in the sample well. If the Ct value for the N-IC is above the upper cut-off value, the Worksheet will state Insufficient Sample Input (if BRAF) for all targets in the sample well. If the Ct value for the B-IC falls between the cut-off values, then it is reported as Sample Input Sufficient and each mutation target is evaluated.

Note: If an input higher or lower than the recommended 10ng is used, B-IC may report Dilute (if BRAF) or Insufficient Sample Input (if BRAF), respectively.

Mutation Targets: If a mutation target does not have a Ct value reported, then Mutation Not Detected is reported for that mutant target. Each mutation target that has a Ct value reported is evaluated by calculating the delta Ct value ($\Delta Ct = Ct \text{ B-IC} - Ct \text{ mutation target}$). The calculated delta Ct value is then compared to the delta Ct cut-off value for that mutation target (See Table 6). If the delta Ct value is outside the cut-off value, then Mutation Not Detected is reported for that mutant target. If more than one mutant target in the sample passes the delta Ct cut-off values, the highest delta Ct value (lowest Ct value) is reported as Mutation Detected, while all other mutant targets are reported as Mutation Not Detected. If two or more targets have the highest delta Ct value, it is reported as Re-run Sample.

In case of a target detection within specifications, the Ct value will be displayed. Table 6 contains the individual cut-off filter for each target.

Table 6: Modaplex BRAF Mutation Analysis Assay Cut-Offs

		Targets	Ct Value Range/ delta Ct
Assay Target	B-IC	B-IC	31,5-32
Mutation Target	BRAF	V600D	-7,5
		V600E	-3,75
		V600E-TG/AA	-7,8
		V600K	-6

Results

The Result tab of the Excel Meta-analysis Worksheet summarize samples and run controls of KRAS/NRAS and BRAF.

Table 7: Modaplex KNB* Summary Sheet (Run controls)

	Run control Pass	Run control Failed
B-PC	BRAF Positive Control	QC Failed
B-NTC	BRAF Negative Control	QC Failed

*KRAS/NRAS/BRAF

Table 8: Modaplex KNB* Summary Sheet (samples)

	Sample Input Sufficient	Insufficient Sample Input or Dilute (if KRAS, NRAS or BRAF)
BRAF Sample name	BRAF Mutation Detected** or BRAF Mutation Not Detected	QC Failed

*KRAS/NRAS/BRAF

** If more than one mutant target in the sample passes the delta Ct cut-off values, the highest delta Ct value (lowest Ct value) is reported as "Mutation Detected" and all other mutant targets are reported as "Mutation Not Detected".

Note: If "QC Failed" is reported, please check the KNB Summary sheet in detail.

Performance Characteristics

Table 7: Performance characteristics of the Modaplex BRAF Mutation Analysis Kit

Assay Performance	Result/Description										
Primer Specificity	The primer of the Modaplex BRAF Mutation Analysis Kit only amplifies human BRAF sequences without cross-reactions to non-BRAF sequences.										
Sample Material	The Modaplex BRAF Mutation Analysis Kit has been verified using artificial sample material spiked into the FFPE background for each target to be detected. Additionally, pre-characterized DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) colorectal tissue have been tested for use with the Modaplex BRAF Mutation Analysis Kit, if available.										
Input	DNA input: 10 ng										
LOD	<p>The assay sensitivity (Limit of detection) was defined as the lowest amount of mutant template (artificial template) in a wild type FFPE DNA background detected in at least 95% of the reactions tested. The table below shows the verified LoD levels for each of the four targets.</p> <table border="1"> <thead> <tr> <th>Mutation Target</th> <th>% Mutant detected in W</th> </tr> </thead> <tbody> <tr> <td>V600D</td> <td>6</td> </tr> <tr> <td>V600E</td> <td>15</td> </tr> <tr> <td>V600E-TG/AA</td> <td>12</td> </tr> <tr> <td>V600K</td> <td>12</td> </tr> </tbody> </table>	Mutation Target	% Mutant detected in W	V600D	6	V600E	15	V600E-TG/AA	12	V600K	12
Mutation Target	% Mutant detected in W										
V600D	6										
V600E	15										
V600E-TG/AA	12										
V600K	12										

Troubleshooting Guide

The troubleshooting guide is intended to help solve any problems that may arise. See also the Modaplex System User Manual for Instrument and Software Troubleshooting Guidance. The scientists at Biotype GmbH are happy to answer any questions about the information and protocols given in these IFU (for contact information, see the back cover).

Failure	Comments and suggestions
Invalid negative control (B-NTC)	<ol style="list-style-type: none">1. Internal control (IC) was detected in B-NTC. Contamination occurred during the preparation of the PCR. Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after adding the sample to be tested. Do not reseal the plates with the same plate seal. Ensure that the work space and the instruments are decontaminated at regular intervals.2. Check the maintenance interval for all used devices (e.g. pipettes).3. B-NTC well was not labeled properly (i.e. NC instead of B-NTC). Check your Modaplex plate map and modify, if necessary. After this, re-analyze and load again into Excel Meta-analysis Worksheet. Make sure that no "spaces" occur after B-NTC when setting up the plate map.
Invalid positive control (B-PC)	<ol style="list-style-type: none">1. Incomplete detection of targets, calibrators, and/or internal controls. Incorrect handling occurred during the preparation of the PCR and/or positive control. Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. Do not reseal the plates with the same plate seal. Ensure that the work space and the instruments are decontaminated at regular intervals.2. Check the maintenance interval for all devices used (e.g. pipettes).3. B-PC well was incorrectly labeled (i.e. PC instead of B-PC). Correct the name of the well in the plate setup on the Modaplex and re-analyze the run. Ensure that no "spaces" occur after B-PC when setting up the plate map.
The storage conditions for one or more kit components do not comply with the instructions given in 'Reagent Storage and Handling Conditions' on p. 11.	<ol style="list-style-type: none">1. Check the storage conditions and the expiration date (see the kit label). Please use a new kit if the reagents were stored improperly.
The Modaplex BRAF Mutation Analysis Kit is past the expiration date.	<ol style="list-style-type: none">1. Check the storage conditions and the expiration date (see the kit label). Please use a new kit if the kit or any kit component is past the expiration date.

Limitations

All results obtained with the product must be interpreted within the context of relevant laboratory findings. The results are not to be used for diagnosis.

The product should be used only by specifically instructed personnel who have been properly trained to use the Modaplex platform.

The product is intended for use with the Modaplex platform only.

For optimal results, strict compliance with the Modaplex BRAF Mutation Analysis Kit IFU is required. The dilution of the reagents, other than that described in this IFU, is not recommended and will result in the loss of performance.

It is important that the amount and quality of the DNA in the sample are assessed and adjusted prior to performing a sample analysis using the Modaplex BRAF Mutation Analysis Kit.

Attention should be paid to expiration dates and storage conditions printed on the box and on the labels of all components. Do not use expired or incorrectly stored components.

Warranties and Disclaimer

This product is warranted to perform as described when used in strict conformity with the instructions given herein. The product has been designed for research use only and is to be used solely by qualified professionals. It is the user's responsibility to ensure that a given product is suitable for a given application.

Biotype GmbH provides no other warranty, expressed or implied, and disclaims any implied warranty of merchantability or fitness for a particular purpose. Under no circumstances whatsoever shall Biotype GmbH be liable for any indirect, special, or consequential damage.

References

- [1] W. Qi Li et al., BRAF mutations are associated with distinctive clinical, pathological and molecular features of colorectal cancer independently of microsatellite instability status, *Molecular Cancer*, 5:2, 2006
- [2] T. J. Semrad, E.J. Kim, Molecular testing to optimize therapeutic decision making in advanced colorectal cancer, *Journal of gastrointestinal oncology* 7, pp. 11–20, 2016.

Symbols

The following symbols may appear on the packaging and labeling:



Contains reagents sufficient for 50 reactions



Expiration date



Catalogue number



Lot number



Temperature limitation



Manufacturer

Technical Assistance

For technical assistance or information, please call Biotype GmbH at +49-(0)351-8838 400.

Contact Information

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Ordering Information

Product	Contents	Cat. no.
Modaplex BRAF Mutation Analysis Kit	For 50 reactions: Primer Mix BRAF, PCR Buffer 2, Modaplex Enhancer 50, Modaplex Polymerase B, Positive Control BRAF, Nuclease Free Water, 10X Injection Buffer, Modaplex Calibrator 2	85-10601-0050
Modaplex and Consumables		
Modaplex System	Modaplex Instrument, Monitor, Barcode Reader Kit, PC Keyboard, PC Mouse, Printer, Cables, 1 Modaplex TC Hold-down Plate, 1 Modaplex Cartridge with 48 Capillaries	00-04901-0001
Modaplex TC Hold-down Plate	1 Modaplex TC Hold-down Plate	00-04X01-0001
Modaplex 48 Capillary Cartridge	1 Modaplex Cartridge with 48 Capillaries	00-14X02-0001
Modaplex Buffer	2 Bottles of Modaplex Buffer	00-14302-2000
Modaplex Decon	2 Bottles of Modaplex Decon	00-14303-2000
POP-7/ Modaplex CE Gel	1 Bottle of Modaplex CE Gel	00-04305-0028
Modaplex Wash	1 Bottle of Modaplex Wash	00-14304-0250
Modaplex CE Plates	1 Box of 20 Modaplex CE Plates	00-04306-0020
Mineral Oil	5 Bottles of Mineral Oil	00-04301-0025
PCR Microplates 96	1 Box of 25 96-well PCR Plates	00-04X03-0025
Aluminum Sealing Film	1 Box of 100 Aluminum Sealing Film	00-04X04-0100

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