Modaplex POLE/POLD1 Mutation Analysis Kit
IFU - Instructions for Use

REF BTI-C003-C1-2-0050

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RUO

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

Intended for use with the Modaplex System.

BIOTYPE

Content

Intended Use **3**
Summary and Explanation **3**
Principle of the Procedure **4**
Kit format and components 4
Control concept 5
Platform and software 6
Kit contents 6
Packaging inserts 7
Materials required (not included in the kit) 9
Warnings and Precautions **9**
Safety Information **10**
General Precautions **10**
Reagent Storage and Handling **11**
Procedure **11**
DNA purification 11
DNA quantification 11
Protocol: Preparing the Modaplex run for POLE/POLD1 Mutation Analysis 12
Protocol: Setting up the POLE/POLD1 Mutation Modaplex run 13
Interpretation of the Results **16**
Data transfer to the Moda-RA software 16
Data analysis 17
Creating a POLE/POLD1 report 25
Performance Characteristics **26**
Troubleshooting Guide **27**
Limitations **28**
Warranties and Disclaimer **28**
References **28**
Symbols **29**
Technical Assistance **29**
Contact Information **29**
Ordering Information **30**

Intended Use

The BTI Modaplex POLE/POLD1 Mutation Analysis Kit is a qualitative and comprehensive PCR-based multiplex assay for the detection of 20 single nucleotide mutations within the exonuclease domain of POLE and POLD1 genes 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 procedures.

Summary and Explanation

POLE and POLD1 are human genes encoding the catalytic subunit (exonuclease domain) of DNA polymerase Epsilon and Delta 1. The main function of POLE/POLD1 exonuclease domains (EDMs) is their proofreading activity—an exonuclease function that detects and removes misincorporated bases in the daughter strand through failed complementary pairing with the parental strand.[1]

Mutations of the exonuclease domains lead to impaired proofreading during the DNA replication and results in a dramatically increased mutation rate. [3] This mutation rate "creates" neo-antigens. It is described that POLE/POLD1 mutant tumors have 15 times more neo-antigens compared to MSI tumors and 100 times more neo-antigens compared to Microsatellite Stable (MSS) tumors.[1] They indicate the presence of tumor-infiltrating, cancer-fighting T-Cells (immune system "readiness"), which are silenced by PD-L1 – PD-1 interactions. There is substantial evidence that these somatic (and rare germline) exonuclease domain mutations are involved in cancer tumorigenesis. It has already been reported that somatic POLE mutations are risk factors for colorectal cancer (CRC) and other tumors like endometrial cancer.[3,4] Polymerase mutations may be the new molecular marker to identify hypermutated colorectal cancer tumors that might respond to immune checkpoint inhibitors beyond deficient mismatch repair (dMMR) CRC.[2] The detection of POLE/POLD1 mutations, in addition to Microsatellite instability (MSI), allows the identification of patients who could benefit from immune checkpoint therapies.

The Modaplex POLE/POLD1 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 20 POLE/POLD1 mutations are amplified using fluorescent-labeled primers that are separated by CE and analyzed through qualitative endpoint detection using the Moda-RA (Modaplex Result Analyzer) software.

The functionality of the assay is controlled by internal and external controls. Table 1 summarizes the list of POLE/POLD1 mutation targets as well as the internal controls.

Table 1: List of assay targets

Position	Target Gene	AA Change	CDs Mutation
1	POLE	T278M	c.833C>T
		P286S	c.856C>T
		P286L	c.857C>T
		P286H	c.857C>A
		P286R	c.857C>G
		S297A	c.889T>G
		S297F	c.890C>T
		F367S	c.1100T>C
		V411L (G>C)	c.1231G>C
		V411L (G>T)	c.1231G>T
		H422N	c.1264C>A
		L424V	c.1270C>G
		P436R	c.1307C>G
		M444K	c.1331T>A
		A456P	c.1366G>C
		S459F	c.1376C>T
		A465V	c.1394C>T

Continue Table 1. List of assay targets

Position	Target Gene	AA Change	CDs Mutation
2	POLD1	D316N	c.946G>A
		C319Y	c.956G>A
		S478N	c.1433G>A
Position	**Controls**	**AA Change**	**CDs Mutation**
3	IC1 (POLE)	-	-
	IC2 (POLD1)	-	-

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 POLE/POLD1 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 20 POLE/POLD1 targets and two internal controls. Calibrators of three sizes, which are used for aligning and assigning CE peaks, are added to each test. At the end of the run, the amplified PCR products are sized using the Ct and the cut-off value specific to each POLE and POLD1 target.

Kit format and components

The Modaplex POLE/POLD1 Mutation Analysis Kit contains reagents that can be used to perform 50 reactions. It includes the following components:

Primer Mix POLE/POLD1 Mutation
This tube contains oligonucleotide primers specific to the 20 POLE/POLD1 mutations as well as to two internal control gene targets, as shown in Table 1. Each primer can have either a FAM label or a TYE label, or it can be devoid of any label.

PCR Buffer 3
This solution is optimized to promote enzyme activity for the PCR in the Modaplex POLE/POLD1 Mutation Analysis Kit.

Modaplex Polymerase T
The Modaplex POLE/POLD1 Mutation Analysis Kit contains a Taq DNA Polymerase (5U).

Modaplex Enhancer 50
This solution enhances the PCR reaction and is thus essential for the process.

Control concept

The POLE/POLD1 Mutation Analysis Kit comes with a comprehensive control concept. It consists of 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 POLE/POLD1 Mutation Analysis Kit has been designed to use Calibrator Kit 2. In both the FAM and TYE channels, 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 Calibrator Kit 2 must be added to all sample -, negative control and positive control wells.

Internal Controls
The Modaplex POLE/POLD1 Mutation assay contains two internal controls, IC1 and IC2, which serve as a template-dependent PCR control in sample wells.

External Control 1: Negative Control (NC)
The user needs to set up an NC (no-template control) for each run to assess the potential contamination while setting up the assay. The NC consists of nuclease-free water (H_2O), which is to be used as a template for the NC well.

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

External Control 2: Positive Control (PC)
The Positive Control POLE/POLD1 Mutation contains an artificial template representing each of the 20 POLE/POLD1 mutations and the two internal controls. 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 POLE/POLD1 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.

Biotype's Moda-RA Software
It is strongly recommended that the Modaplex POLE/POLD1 Mutation Analysis Kit be analyzed using Biotype's Moda-RA (**Moda**plex **R**esult **A**nalyzer) software. The software already contains all the information required for the analysis of POLE/POLD1 mutation and no special updates, dongles, or unlocking procedures are required to start the interpretation of the results. Please refer to 'Interpretation of the Results' on p. 17–25 for detailed information on the use of the Moda-RA software for POLE/POLD1 mutation analysis.

Materials Provided

Kit contents

Table 3 summarizes the contents of the Modaplex POLE/POLD1 Mutation Analysis Kit and indicates the required storage conditions.

Table 3: Content of the POLE/POLD1 Mutation Analysis Kit

Component	Ingredients	Vials	Vol./Vial (µL)	Cap Color	Storage
Primer Mix POLE/POLD1 Mutation	a) FAM or TYE-labeled and unlabeled primer for POLE/POLD1mutation b) Control gene primer c) Tris-EDTA buffer	1	125	Red	−15 °C to −25 °C
PCR Buffer 3	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 T	a) Taq polymerase b) Glycerol	2	15	Orange	−15 °C to −25 °C
Positive Control POLE/POLD1 Mutation	a) Control template b) Tris-EDTA buffer	1	10	White	−15 °C to −25 °C
Nuclease Free Water	a) Nuclease-free water	1	1,500	Light blue	−15 °C to −25 °C

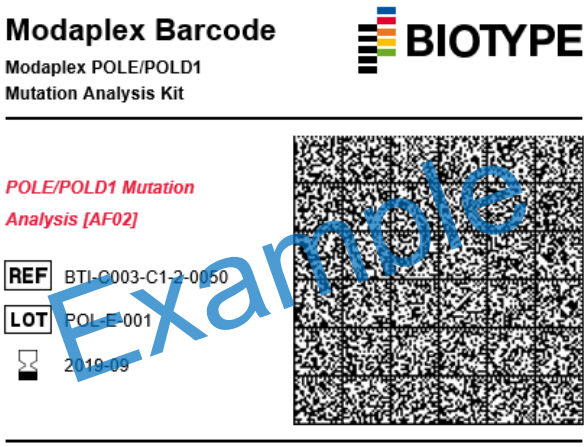
Packaging inserts

The Modaplex POLE/POLD1 Mutation Analysis Kit is delivered with two packaging inserts—the Modaplex Barcodes and the IFU Download Instructions. Each kit must be used with the packaging inserts supplied therein. This guarantees that the test is set up according to the latest instructions for use (IFU) and that the correct Modaplex assay definitions are used for POLE/POLD1 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 POLE/POLD1 Mutation Analysis Kit, this barcode must be scanned. On scanning the barcode, POLE/POLD1-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 and, subsequently, to the Moda-RA software.

Figure 1: Example of Modaplex Barcode



The **Modaplex Barcode** contains all POLE/POLD1 Mutation Kit-related information required for the detection of POLE/POLD1 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 POLE/POLD1 Mutation Analysis kit. With each new lot of the assay, the newly provided barcode needs to be scanned, which results in a new assay definition on the Modaplex instrument, including the new lot number and the expiration date.

Note: In terms of traceability, the Modaplex consumables and assay definition will be present in the Mode-RA report once the barcode has been scanned and a Modaplex run has been performed with the new POLE/POLD1 Mutation Analysis Kit.

Note: The Moda-RA configuration identifier is shown in square brackets behind the assay name. This unique code enables the analysis of the results in the Moda-RA.

Modaplex IFU Download

The POLE/POLD1 Mutation IFU is not physically a part of the Modaplex POLE/POLD1 Mutation Analysis Kit. However, to guarantee that the kit is used according to the dedicated IFU, the POLE/POLD1 Mutation assay comes with a packaging insert labeled `Modaplex IFU Download'. It provides guidance about 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 POLE/POLD1 Mutation webpage using the link given on the Modaplex IFU download packaging insert: https://www.biotype-innovation.de/product/ici/pole-pold1/

2. **Click** on the respective IFU link in the POLE/POLD1 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 at support@biotype.de and request the dedicated IFU.

Materials required (not included in the kit)

In addition to the kit content, the following reagents, consumables, and equipment are required but not provided with the Modaplex POLE/POLD1 Mutation Analysis Kit.

Reagents and Consumables

- Modaplex Calibrator Kit 2 (Biotype)
- 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)
- Aluminum Sealing Film (Biotype)
- Modaplex CE 48 (Catridge) (Biotype)
- Modaplex Buffer (Biotype)
- Modaplex Decon (Biotype)
- Modaplex Wash (Biotype)
- POP -7/ Modaplex CE Gel (Thermofisher)
- Modaplex CE Plates (Biotype)

Instruments and Software

- Modaplex System (Biotype)
- Moda-RA Analysis Software (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

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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 POLE/POLD1 Mutation Analysis Kit components, please consult the corresponding safety data sheets (SDS), which are available upon request. For the safety information of any component not provided with the kit, please contact the respective supplier.

Modaplex Safety Information

Due to the high voltage required for the CE separation, failure to fill all wells is a general safety threat that may cause damage to the Modaplex instrument.

General Precautions

The user should always 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 equal 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 issues, separate the different procedure 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 set-up. 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 POLE/POLD1 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 POLE/POLD1 Mutation Analysis Kit. In order to maintain optimal performance, the reagents in the kit must not be substituted.

Note: Use only the Taq DNA polymerase (Modaplex Polymerase T) 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 POLE/POLD1 Mutation Analysis Kit is shipped on dry ice. Upon receipt, the assay should immediately be stored at −15 °C to −25 °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 POLE/POLD1 Mutation Analysis Kit immediately 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 POLE/POLD1 Mutation Analysis Kit has been verified using artificial sample material spiked into the FFPE background for each target to be detected. In addition, pre-characterized DNA samples (mutations A456P and P286R) extracted from formalin-fixed paraffin-embedded (FFPE) endometrial and colorectal tissue have been tested for use with the Modaplex POLE/POLD1 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 POLE/POLD1 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

Quantification of the DNA should be carried out 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 POLE/POLD1 Mutation Analysis Kit using 4ng (0,8ng/µl). Using a DNA amount below 4ng will result in low PCR yields and the signal might fall below the target-specific detection limits. Using a DNA amount above 4ng could cause signal background and therefore wrong positive results.

Protocol: Preparing the Modaplex run for POLE/POLD1 Mutation Analysis

This protocol is intended for the preparation of the Modaplex instrument prior to setting up the Modaplex POLE/POLD1 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 (NC)
- 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 POLE/POLD1 mutation assay definitions to the Modaplex instrument.

Scan the two Modaplex barcodes provided with the Modaplex POLE/POLD1 Mutation Analysis Kit. The assay definitions are automatically added to the Modaplex System. See the Modaplex System User Manual for further instructions.

Note: The Modaplex barcodes need to be scanned every time a new lot of the Modaplex POLE/POLD1 Mutation Analysis Kit is used.

4. Confirm the Modaplex settings for the POLE/POLD1 Mutation Modaplex run.

Before setting up the Modaplex POLE/POLD1 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 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 Moda-RA software requires the following form of **sample-naming:**

- Positive control: assay name_PC
- Negative control: assay name_NC
- 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 like double quotes (“), brackets (< or >), ampersands (&), etc.

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

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

Protocol: Setting up the POLE/POLD1 Mutation Modaplex run

This protocol is to be used for the preparation of reagents in the Modaplex POLE/POLD1 Mutation Analysis Kit and the PCR plate for the Modaplex run.

1. **Remove and thaw** the following components from the Modaplex MSI Analysis Kit:
   - PCR Buffer 3 (black cap)
   - Modaplex Enhancer 50 (natural cap)
   - Primer Mix POLE/POLD1 (red cap)
   - Nuclease Free Water (light-blue cap)
   - Positive Control POLE/POLD1 (white cap)
   - Modaplex Polymerase T (orange cap)

   Note: The Modaplex Polymerase T is temperature-sensitive. Keep the enzyme at −15 °C to −25 °C at all times.

2. **Remove and thaw** the following components from the **Calibrator 2** kit**:**
   - Calibrator 2 (yellow cap)
   - 10x injection buffer (blue cap)

3. **Homogenize** the thawed reagents by inverting the tubes, pipetting, or gently vortexing. After this, briefly centrifuge the reagents.

4. **Preparing the POLE/POLD1 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: POLE/POLD1 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 3	12.5 µL	25.0 µL	37.5 µL	50.0 µL	62.5 µL	125.0 µL
Primer Mix POLE/POLD1	2.5 µL	5.0 µL	7.5 µL	10.0 µL	12.5 µL	25.0 µL
Nuclease Free Water	2.0 µL	4.0 µL	6.0 µL	8.0 µL	10.0 µL	20.0 µL
Modaplex Enhancer 50	1.5 µL	3.0 µL	4.5 µL	6.0 µL	7.5 µL	15.0 µL
Modaplex Polymerase T	0.5 µL	1.0 µL	1.5 µL	2.0 µL	2.5 µL	5.0 µL
Calibrator 2	1.0 µL	2.0 µL	3.0 µL	4.0 µL	5.0 µL	10 µL
Total Volume of Master Mix (MMx)	**20 µL**	**40 µL**	**60 µL**	**80 µL**	**100 µL**	**200 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 POLE/POLD1 master mix to the designated wells in the PCR plate.

7. **Add 5 µL** of each of the following:
   - Extracted DNA (template, optimum 4 ng) to the corresponding sample well(s)
   - Nuclease Free Water to the NC well
   - Positive Control POLE/POLD1 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 µL** of **1x injection buffer (1x IB)** to the remaining empty wells that are not being used for a sample well, NC or PC.

- Prepare a 1:10 dilution of the 10x injection buffer with water.
- Add 25 µL of the 1x injection buffer to each of the empty wells. Please refer to the plate map set-up in the Modaplex System IFU for further explanation.

Due to the high voltage required for the CE separation, the failure to 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 13 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	POL_PC MMx	Empty 1x IB	Empty 1x IB						
H	Sample 08 MMx	Sample 16 MMx	Sample 24 MMx	POL_NC 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 reaction 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 they are placed on the Modaplex instrument.

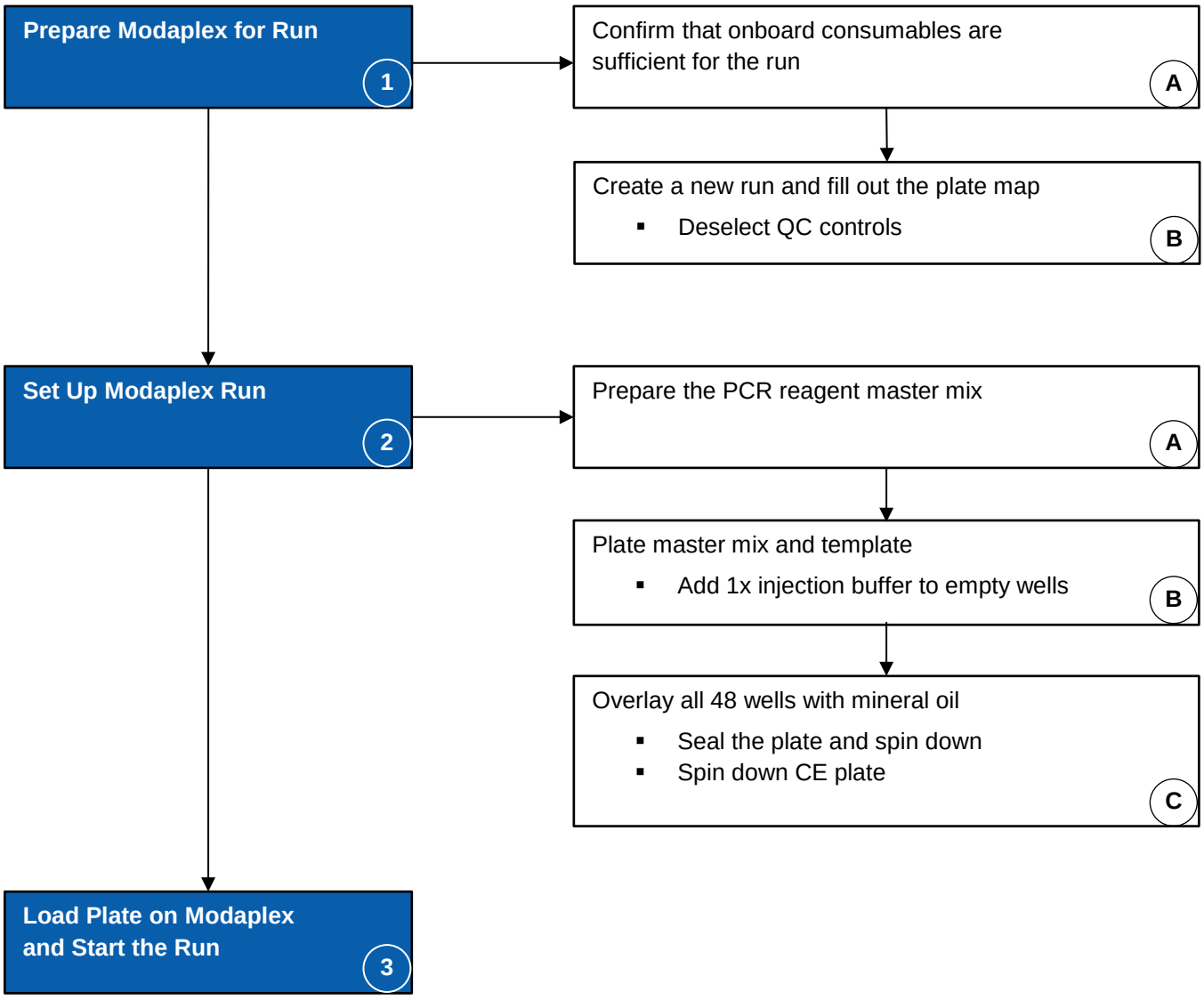
12. **Run the PCR plate** in the Modaplex instrument.

13. **End of Run**

At the end of the run, **seal** the PCR and CE plates with aluminum sealing film before disposing of 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 POLE/POLD1 data must be analyzed using the following procedure:

1. Data transfer to the Moda-RA software
2. Data analysis
3. POLE/POLD1 report creation

Data transfer to the Moda-RA software

The following section describes how the POLE/POLD1 data is transferred from the Modaplex system to the Moda-RA software.

Before analyzing the Modaplex data for the first time, move the folder containing the Moda-RA software onto your computer. Additionally, a local folder should be created on the computer in which all the Modaplex runs are saved.

Note: The Moda-RA software should not be installed on the Modaplex instrument.

1. **Move** the Modaplex **run file to your analysis computer.**

After the completion of the Modaplex run, copy the relative run file '*RunXXXX*' to an external device and paste it in the folder created on your analysis computer.

Note: The Modaplex instrument creates a run folder for each Modaplex run, containing all the run-related data. The folder is automatically generated and named with a consecutive number, e.g. Run0001, Run0002, or Run003. The relative run folder '*Run0001*' is saved in the folder '*Modaplex Data*', which is present as a shortcut on the desktop of the Modaplex computer.

2. **Open** the Moda-RA software by double-clicking on the Moda-RA.exe file.

3. **Select** *Import Modaplex Run*.

4. **Select** your run folder.

5. **Select** *Finish*.

This loads the Modaplex data into the Moda-RA software. The quality control for all wells is performed automatically during import. After the data transfer, the '*Run Overview*' window opens for data analysis.

Data analysis

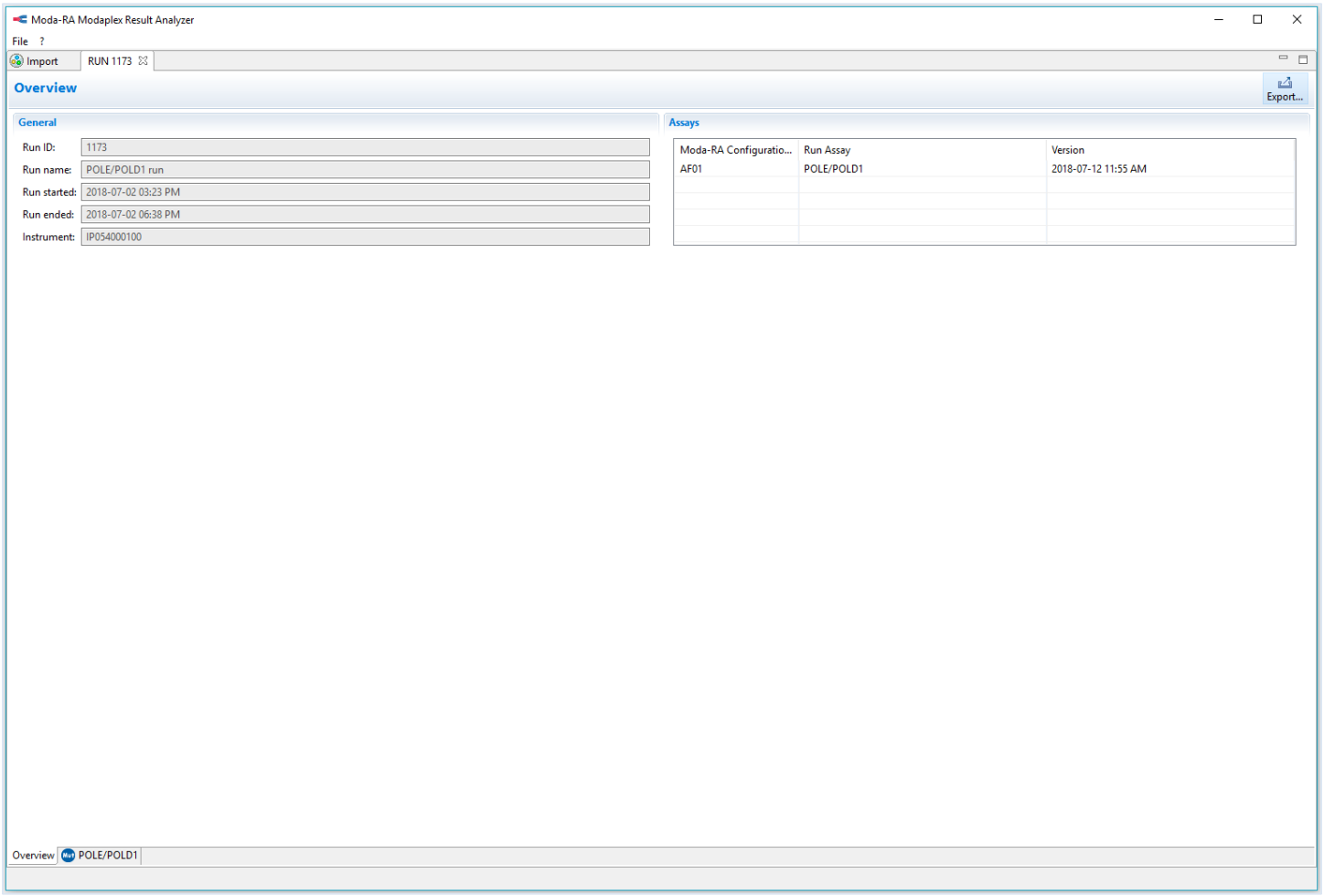
The following section describes how the Moda-RA software is used for the analysis of the POLE/POLD1 data and how the results are displayed. This section also contains detailed information about the result interpretation of the control and the sample wells, including the comments and recommendations pertaining to all possible results. This section is divided into the following subsections:

A. Run overview
B. Sample plate summary and general display of analyzed data
C. Quality control: Analysis of the NC and PC
D. Result interpretation: Analysis of the sample wells

A. Run overview

The "*Overview*" window opens automatically after the data import is completed. It contains the Modaplex-related run information, such as run ID, run name, start of run, end of run and the Modaplex instrument identifier. In addition, the Moda-RA configuration identifier is displayed along with the associated assay. All assays present on the plate are displayed.

Figure 4: Example of the "Overview" window



B. Plate summary and display of the analyzed data

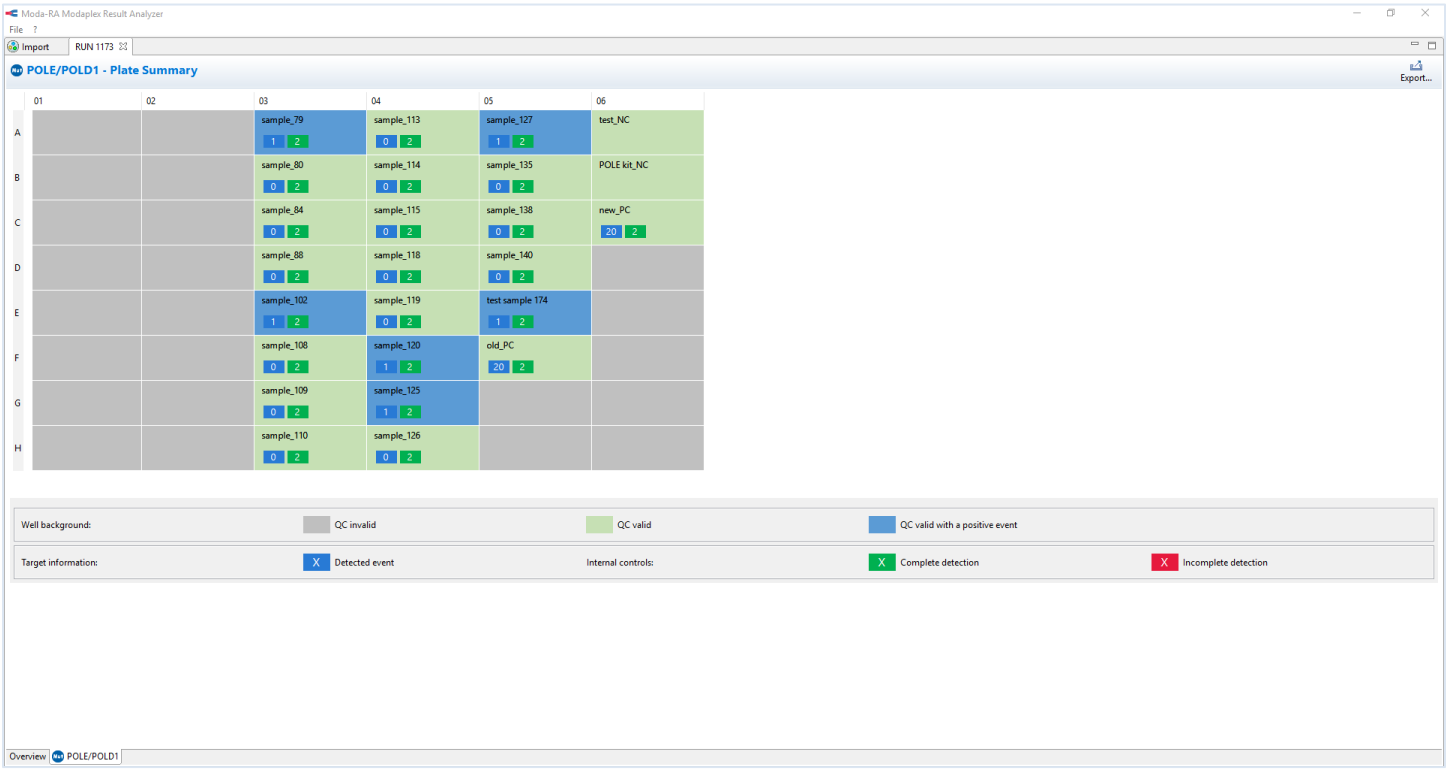
The '*POLE/POLD1—Plate Summary*' window can be selected by clicking on the '*POLE/POLD1*' tab at the bottom of the window. The plate map shows all POLE/POLD1 controls and sample wells present on the well.

A color code is used to display the validity of each well. The applied color code displays the following information:

1. QC information: Independent determination of the validity of each well
2. Internal control information: The number of the detected internal controls

The plate summary is illustrated in Figure 5. The color code is explained in detail in Figure 6.

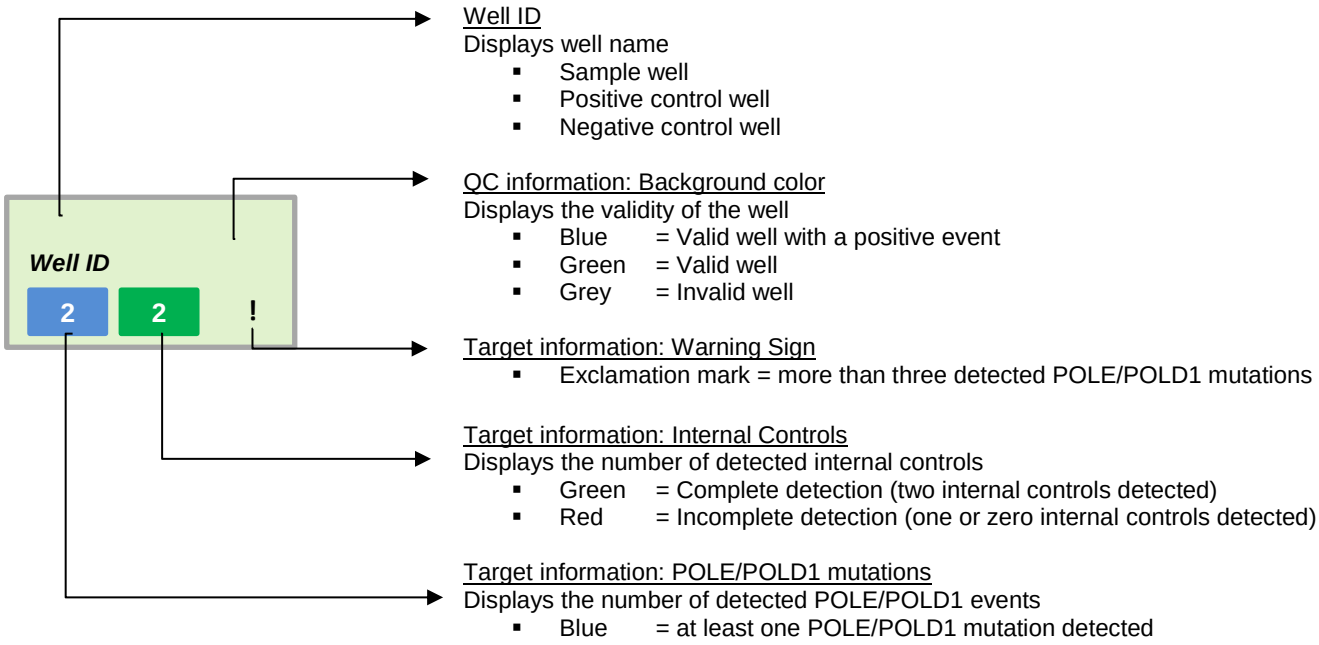
Figure 5: Illustration of the plate summary



Note: All wells are reported except the empty wells.

Note: If another assay is set up and run on the same PCR plate, the results are shown in a separate tab.

Figure 6: Explanation of the Moda-RA color code



Note: Detailed information on the interpretation of the control and the sample wells is given in Tables 5–9.

C. Quality control: The analysis of the NC and PC

Each Modaplex POLE/POLD1 run should include the run controls NC and PC. A Modaplex run is valid if the PC and the NC wells are valid. The results of the run controls are displayed through changes in the background color of each well on the plate summary.

Note: The validity of the run controls is not connected to the validity of the sample wells. A sample well can be valid even when there are invalid run controls. In such cases, the reason behind the invalid controls should be investigated and Biotype GmbH should be contacted for technical assistance, if required.

1. Negative Control (NC)

The NC is reported as valid if no internal control (IC1 and IC2) or target is detected. It is reported as invalid if at least one internal control or target is detected. Please review the Biotype GmbH troubleshooting guide in case of a failed NC.

Note: For more detailed information, please refer to Table 5.

2. Positive Control (PC)

The PC is reported as valid if all markers and internal controls are detected. If the PC fails, the user should review the PC by consulting the Biotype GmbH troubleshooting guide.

Note: Please refer to Tables 5, 6, and 7 for detailed information on the criteria for valid or invalid run controls.

Table 5: Moda-RA: Validity of PC and NC wells

Background color of control wells	Description	Criteria
	• Valid Modaplex run for PC and NC wells	• At least 2 out of 3 calibrator peaks detected in NC or PC (calibrator 1 must be present) • All targets detected in PC • All internal controls detected in PC • No target detected in NC • No internal controls detected in NC
	• Invalid Modaplex run for PC and NC wells	• Calibrator 1 not detected in NC or PC • Calibrator 2 and 3 are not detected • Less than 20 targets detected in PC • No internal controls detected in PC • Targets detected in NC • Internal controls detected in NC

Note: In the case of a systematic Calibrator 2 kit failure, all control wells (NC and PC) become invalid and are therefore colored grey. It is recommended to set up the run again using a new Calibrator 2 kit.

Table 6: Moda-RA software: Detection of the internal controls

Color and number code for the positive control	Description	Criteria
2	• Complete detection of internal control	• Both internal controls detected
1	• Incomplete detection of internal controls	• One internal control detected—IC1 or IC2
0	• Incomplete detection of internal controls	• No internal control detected

Note: No small boxes are shown for NC wells as no targets or internal controls should be detected.

Table 7: *Moda-RA software: Number of POLE/POLD1 targets in PC*

Color and Number Code for POLE/POLD1 targets	Description
20	• Displays the number of detected POLE/POLD1 targets (0–20)

D. Result Interpretation: Analysis of the sample wells

The '*POLE/POLD1* window displays the background colors on the panel summary. The Moda-RA software automatically analyzes the wells and categorizes them as either valid or invalid. Tables 8, 9, and 10 describe the color coding in detail.

Table 8: Moda-RA software: The QC information for the sample wells

Background Color of sample wells	Description	Criteria
	• Valid sample well	• At least two out of three calibrator peaks detected (Calibrator peak 1 must be present) • Internal controls IC1 and IC2 detected
	• Valid sample well	• At least two out of three calibrator peaks detected (Calibrator peak 1 must be present) • Internal controls IC1 and IC2 detected • At least one POLE/POLD1 target detected within assay specifications
!	• Warning Sign shown for a highly unlikely detection of more than three POLE/POLD1 mutations	• ≥3 POLE/POLD1 targets detected within assay specifications • Please refer to p. 19 of the troubleshooting section.
	• Invalid sample well	• Calibrator 1 not detected in sample well • Calibrators 2 and 3 not detected • Fewer than two internal controls detected

Table 9: Moda-RA software: Detection of the internal controls

Color and number code for internal controls in sample wells	Description	Criteria
2	• Complete detection of internal control	• Both internal controls detected
1	• Incomplete detection of internal controls	• One internal control detected—IC1 or IC2
0	• Incomplete detection of internal controls	• No internal control detected

Table 10: Moda-RA software: Detection of targets

Color and Number Code for POLE/POLD1 Targets (examples) in sample wells	Description	Criteria
0	• Displays the number of detected POLE/POLD1 targets	• Detection within assay specifications

In case of a target detection within specifications, the Ct value will be displayed. Table 11 contains the individual cut-off filter for each target.

Table 11: Cut-off filter for POL/POLD1 mutation targets and sample controls

	Targets	Ct cut-off filter individual for each POLE/POLD1 target			
POLE	T278M	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	P286H	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	P286L	Ct < 19.0	19.0 < Ct <32.5	32.5 < Ct <33.0	Ct ≥ 33.0
	P286R	Ct < 19.0	19.0 < Ct <33.5	33.5 < Ct <34.0	Ct ≥ 34.0
	P286S	Ct < 19.0	19.0 < Ct <33.5	33.5 < Ct <34.0	Ct ≥ 34.0
	S297A	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	S297F	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	F367S	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	V411L(G>C)	Ct < 19.0	19.0 < Ct <35.0	35.0 < Ct <36.0	Ct ≥ 36.0
	V411L(G>T)	Ct < 19.0	19.0 < Ct <35.0	35.0 < Ct <36.0	Ct ≥ 36.0
	H422N	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	L424V	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	P436R	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	M444K	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	A456P	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	S459F	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	A465V	Ct < 19.0	19.0 < Ct <35.0	35.0 < Ct <36.0	Ct ≥ 36.0
POLD1	D316N	Ct < 19.0	19.0 < Ct <35.0	35.0 < Ct <36.0	Ct ≥ 36.0
	C319Y	Ct < 19.0	19.0 < Ct <35.0	35.0 < Ct <36.0	Ct ≥ 36.0
	S478N	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
Samples controls	IC1	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
	IC2	Ct < 19.0	19.0 < Ct <34.0	34.0 < Ct <35.0	Ct ≥ 35.0
Interpretation		Failed POLE/POLD1 mutation or reference detection due to high sample input	Optimal Ct detection for POLE/POLD1 mutation and internal controls	Weak detection of POLE/POLD1 mutation or sample control signal	No detection of POLE/POLD1 mutation or internal control

A Ct value outside the optimal range and outside the range for weak signals indicates the absence of POLE/POLD1 mutations if the Ct values of the internal controls IC1 and IC2 are within the optimal range. The run failed if the Ct values are outside their optimal range and if Ct values are outside the range for weak signals for POLE/POLD1 mutations and internal controls. It is recommended to repeat the Modaplex run with a greater amount of sample DNA.

For more information about the well, **move the cursor** above the well.

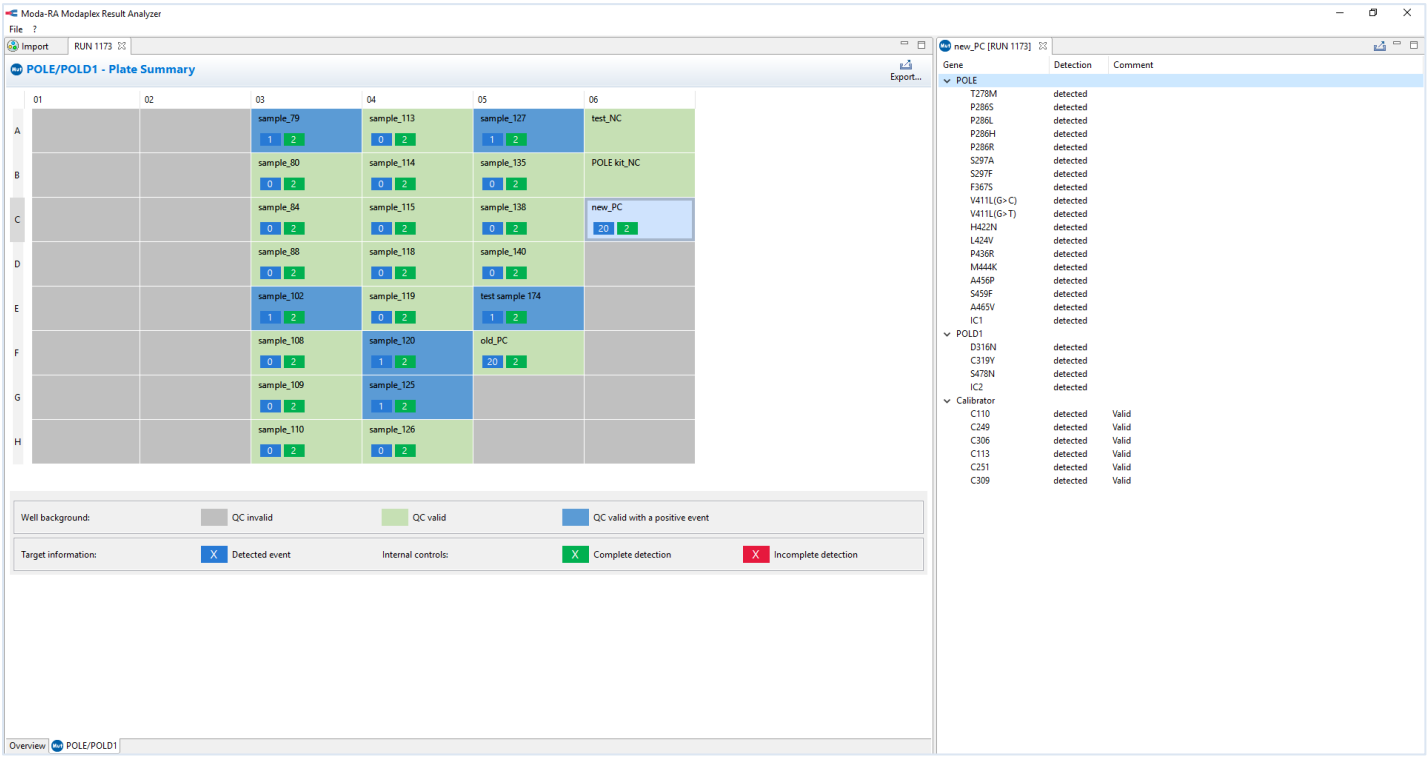
1. A pop-up window appears, displaying well name and well type.
2. **Double-click** the well of interest to see the results.

   A tab opens on the right of the well. A table containing the target panel and the calibrators is displayed:

   - Genes: POLE, POLD1, and calibrator
   - Detection of marker and calibrators
   - Ct values for detected targets and internal controls

The well tab is illustrated in Figure 7.

Figure 7: Illustration of detailed well information (opens on the right for every well)



Note: For detected POLE/POLD1 mutations and the internal controls (IC1 and IC2), Ct-values will be displayed in sample wells without corresponding comments. No Ct values will be displayed in positive control wells.

Creating a POLE/POLD1 report

This protocol is to be used for the preparation of a report after the POLE/POLD1 data has been analyzed and classified as valid. The report contains the following information:

- The result of the sample
- Sample information
- Modaplex POLE/POLD1 Mutation Analysis Kit information, such as lot, assay ID, and expiration date
- Information on the Modaplex run, including consumable lots

For creating the POLE/POLD1 report and exporting the report as a *.pdf file, the user should follow the instructions below:

1. **Select** *File* on the Toolbar of the '*Plate Summary*' window and click on *Export.*

2. **Select** *Create a Printable Report* and then select *Next*.

3. **Choose** the file export destination.

   In this window, it is possible to include the calibrator lot number on the report.

4. **Select** *Finish.*
   A *.pdf file is created automatically.

To create a POLE/POLD1 in a *.csv file format, the user should follow the instructions below:

1. **Select** *File* on the Toolbar of the '*Plate Summary*' window and click on *Export.*

2. **Select** *Export to files* and then select *Next*.
   Now you can choose from various csv-format options as well as the sylk-format.

3. **Choose** the folder where you want to save your file.

4. **Select** *Finish.*
   The file is created automatically.

   Note: The document will be automatically named `*POLE POLD1*` and should be renamed after saving.

Performance Characteristics

Table 12: ***Performance characteristics of the Modaplex POLE/POLD1 Mutation Analysis Kit***

<table>
<tr><th>Assay Performance</th><th>Result/Description</th></tr>
<tr><td>Primer Specificity</td><td>A Basic Local Alignment Search Tool (BLAST) analysis has been performed. The primer of the Modaplex POLE/POLD1 Mutation Analysis Kit only amplifies human POLE/POLD1 sequences without cross-reactions to non-POLE/POLD1 sequences.</td></tr>
<tr><td>Sample Material</td><td>The Modaplex POLE/POLD1 Analysis Kit has been verified using artificial sample material spiked into the FFPE background for each target to be detected. In addition, pre-characterized DNA samples (mutations A456P and P286R) extracted from formalin-fixed paraffin-embedded (FFPE) endometrial and colorectal tissue have been tested for use with the Modaplex POLE/POLD1 Analysis Kit.</td></tr>
<tr><td rowspan="2">Input Range</td><td>DNA input: 2–8 ng</td></tr>
<tr><td>Optimum: 4 ng</td></tr>
<tr><td>LOD</td><td>The assay sensitivity (Limit of detection) was defined as the lowest amount of mutant template (artificial template) in a wild type FFPE DNA background that was detected in at least 95% of the reactions tested. The table below shows the verified LoD levels for each of the 20 targets.
<table>
<tr><th>Mutation Target</th><th>% Mutant detected in W</th></tr>
<tr><td>T278M</td><td>25%</td></tr>
<tr><td>P286S</td><td>10%</td></tr>
<tr><td>P286R</td><td>25%</td></tr>
<tr><td>P286L</td><td>25%</td></tr>
<tr><td>P286H</td><td>5%</td></tr>
<tr><td>S297F</td><td>10%</td></tr>
<tr><td>S297A</td><td>>25%</td></tr>
<tr><td>L424V</td><td>25%</td></tr>
<tr><td>S478N</td><td>10%</td></tr>
<tr><td>C319Y</td><td>10%</td></tr>
<tr><td>D316N</td><td>25%</td></tr>
<tr><td>A456P</td><td>5%</td></tr>
<tr><td>S459F</td><td>5%</td></tr>
<tr><td>V411L(G>C)</td><td>10%</td></tr>
<tr><td>V411L(G>T)</td><td>35%</td></tr>
<tr><td>F367S</td><td>10%</td></tr>
<tr><td>A465V</td><td>10%</td></tr>
<tr><td>H422N</td><td>25%</td></tr>
<tr><td>M444K</td><td>10%</td></tr>
<tr><td>P436R</td><td>25%</td></tr>
</table></td></tr>
</table>

Troubleshooting Guide

The troubleshooting guide may be helpful for solving 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 (_NC)	1. Targets and/or internal controls were detected in NC. Contamination occurred during the preparation of the PCR. 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 used devices (e.g. pipettes) 3. NC well was not labeled properly (i.e. NC instead of _NC). Check your Modaplex plate map and modify, if necessary. After this, re-analyze and load again into Moda-RA. Please make sure that no “spaces” occur after _NC when setting up the plate map.
Invalid positive control (_PC)	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 used devices (e.g. pipettes) 3. PC well was incorrectly labeled (i.e. PC instead of _PC). Correct the name of the well in the plate set-up on the Modaplex and re-analyze the run. Please make sure that no “spaces” occur after _PC when setting up the plate map.
Pop up window appears in case of detection of three or more POLE/POLD1 targets	1. Detection of more than three targets is high unlikely. Please make sure that no contamination occurred in your well. In addition, please call the technical service (for contact information, see the back cover).
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	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 POLE/POLD1 Mutation Analysis Kit is past the expiration date	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 all relevant laboratory findings. The results are not to be used for diagnosis.

The product is to 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 POLE/POLD1 Mutation Analysis Kit IFU is required. The dilution of the reagents, other than as described in this IFU, is not recommended and will result in 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 POLE/POLD1 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] S. Brigs and I. Tomlinson. "Germline and somatic polymerase ε and δ mutations define a new class of hypermutated colorectal and endometrial cancers", J. Pathol, 230: pp. 148–153, 2013.

[2] C. Tournigand et al., "Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency.", Crit Rev Oncol Hematol 113, pp. 242–248, 2017.

[3] Castellucci et al., 2017: "DNA Polymerase ε Deficiency Leading to an Ultramutator Phenotype: A Novel Clinically Relevant Entity": Oncologist, 22(5):497-502.

[4] C. Billingsley et al., "Polymerase ε (*POLE)* mutations in endometrial cancer: Clinical outcomes and implications for Lynch Syndrome testing.", Cancer, pp. 386–394, 2016.

Symbols

The following symbols may appear on the packaging and labelling:

 <50 >	Contains reagents sufficient for 50 reactions (25 pairs)
	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.

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Ordering Information

Product	Contents	Cat. no.
Modaplex POLE/POLD1 Mutation Analysis Kit	For 50 reactions: Primer Mix POLE/POLD1, PCR Buffer 3, Modaplex Enhancer 50, Modaplex Polymerase T, Positive Control POLE/POLD1, Nuclease-free Water	BTI-C003-C1-2-0050
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 CE 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	00-14306-0020
Mineral Oil	5 Bottles of Mineral Oil	00-04301-0025
Modaplex Calibrator Kit 2	1 Tube Modaplex Calibrator 2 (0.2 mL), 3 Tubes 10X Injection Buffer (1.5 mL each)	00-14502-0200
PCR Microplates 96	1 Box of 25 96-well PCR Plates	00-14X03-0025
Aluminum Sealing Film	1 Box of 100 Aluminum Sealing Film	00-14X04-0100

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