

Modaplex MSI Analysis Kit

IFU - Instructions for Use



BTI-C002-E1-2-0050



50 (25 pairs)



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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 BTI Modaplex MSI Analysis Kit is a qualitative and comprehensive PCR-based multiplex assay for the detection of micro-satellite instability in human DNA derived from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) samples. 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.

The assay is intended for use with the Modaplex instrument.

Summary and Explanation

During replication and recombination, DNA mismatch repair (MMR) gene products identify and repair the mis-incorporation of bases as well as the short erroneous insertion and deletion loops ⁽¹⁾. Irreversible mutations in DNA MMR genes such as MLH1, MSH2, PMS2, and MSH6 are associated with a loss of DNA mismatch repair activity and cause genomic instability. A prominent consequence of a damaged DNA repair system is that repetitive sequences known as microsatellites become unstable and vary in length ⁽²⁾. Thus, the presence of microsatellite instability (MSI) represents evidence that MMR is impaired ⁽³⁾.

Detection of MSI has become a routine application ever since it was first observed and described for HNPCC patients in 1998 ⁽⁴⁾. Following these observations, the National Cancer Institute (NCI) has published two guidelines—the Bethesda Guidelines ⁽⁵⁾ in 1998 and in 2004 the Revised Bethesda Guidelines ⁽⁶⁾. The NCI recommends MSI testing for the identification of germline MMR damage, associated with the Lynch syndrome, using a set of mono- and dinucleotide markers or a set of five quasi-monomorphic microsatellite markers.

In 2015 it was shown that patients suffering from CRC with a deficient mismatch repair system are susceptible to immune checkpoint blockade ⁽⁷⁾. Since then, there is ongoing discussion regarding the suitability of MSI as a biomarker for predicting the clinical benefits of immune checkpoint inhibition.

The Modaplex MSI Analysis Kit is a fluorescent PCR-based multiplex assay intended for use with the Modaplex platform. With a combination of three dinucleotide repeat markers (D2S123, D5S346, D17S250) and five quasi-monomorphic mononucleotide markers (Bat-25, Bat-26, NR-21, NR-24, Mono27), the assay facilitates the identification of the MSI-H status according to the aforementioned standardized Bethesda or Revised Bethesda Guidelines.

Table 1 summarizes the list of markers and internal controls.

Table 1: List of assay markers

Position	Quasi-monomorphic mononucleotide marker	Gene loci
1	Bat-25	c-kit, intron 16
2	Bat-26	hMSH2 gene, intron 5
3	NR-21	SLC7A8, 5_UTR
4	NR-24	ZNF2, 3_UTR
5	Mono27	MAP4K3, intron 13
Position	Dinucleotide marker	Gene loci
6	D2S123	MSH2
7	D5S346	APC
8	D17S250	Mfd15
Position	Controls	Gene loci
9	HLD131	SHH
10	HLD133	ULK4

Principle of the Procedure

The Modaplex MSI Analysis Kit is to be used with the Modaplex platform. The microsatellite markers are amplified through fluorescent-labelled sequence-specific primer. During PCR, amplification products are injected electrokinetically and separated in an automated CE process. Electrophoresis is performed periodically every second cycle, starting from PCR Cycle 19 and ending with Cycle 41.

The present microsatellite instability is assessed using the **Modaplex Result Analyzer** (Moda-RA) software by comparing the fragment length profiles of matching tumor and normal tissue. A peak size shift in the tumoral sample but not in the normal sample indicates MSI.

Kit format and components

The Modaplex MSI Analysis Kit contains reagents that can be used to perform 50 reactions (25 reaction pairs). It includes the following components:

Primer Mix MSI

This tube contains the oligonucleotide primers specific to eight targets (Bat-25, Bat-26, NR-21, NR-24, Mono27, D2S123, D5S346, D17S250) and two internal controls (HLD131 and HLD133). Each primer can either be labelled with a FAM- or TYE-fluorophore or be devoid of any label.

Modaplex Buffer 3

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

Modaplex Polymerase T

The Modaplex MSI Analysis Kit contains a Taq DNA Polymerase (5U).

Modaplex Enhancer 50

This solution is essential for the process as it enhances the PCR reaction.

Control concept

The Modaplex MSI Analysis Kit comes with a comprehensive control concept. It consists of internal and external controls that are used to evaluate the functionality of the PCR reaction and identify potential contamination. The control concept is described below:

Template-independent PCR control: Calibrators

The Modaplex MSI Analysis Kit has been designed to use the Calibrator Kit 2. In the FAM and TYE channels, three calibrators of different sizes are amplified. They represent a template-independent PCR control and internal length standard. The Calibrator Kit 2 must be added to all sample -, negative control and positive control wells.

Table 2: Calibrator lengths in FAM and TYE channels

	Calibrator lengths FAM channel	Calibrator lengths TYE channel
CAL 1	110	113
CAL 2	249	251
CAL 3	306	309

Note: Calibrator lengths could slightly differ (maximum of 5 bp) between the different calibrator lots used. This variation has no impact on assay analysis or results.

Internal Controls: Human Locus Deletion/Insertion (HLD) polymorphism

These forensically accepted human deletion/insertion polymorphisms support the genetic discrimination of human individuals. The Modaplex MSI assay contains two HLD markers—HLD131 and HLD133. They are amplified within every sample and positive control well serving both as a template-dependent PCR control as well as a sample mix-up control. Additionally, they can be used to identify contamination with human sample material.

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. Nuclease-free water serves as a template for the NC well. In addition, the NC needs to be set up to assess the validity of the whole run. The NC is valid if no band is present at the site of the HLD marker.

Note: The NC includes accompanying bands (peaks) of defined lengths (AB118, AB273, AB266, AB143, AB161) due to technical limitations. These have no impact on the validity of the assay.

External Control 2: Positive Control (PC)

Human gDNA is serves as PC for the MSI assay. Therefore, the PC shows a similar peak pattern as sample well. All targets must be detectable within acceptable ranges, which confirms the proper functioning of the Primer Mix MSI. Consequently, the PC ensures that the kit performs within the stated acceptance criteria.

To confirm the proper functioning of all assay components one PC needs to set up for each run.

Note: The PC does not indicate instability of the microsatellite marker. Instability is a result of polymerase slippage during replication and is genetically indicated by fragment-length shifts. The primer binding site is identical for unstable and stable microsatellite markers.

Platform and software

Modaplex Instrument

The Modaplex MSI 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 GmbH Moda-RA Software

It is strongly recommended that the Modaplex MSI Analysis Kit be analysed using Biotype GmbH's Moda-RA software. The software already contains all the information required for the analysis of microsatellite shifts. No special updates, dongles, or unlocking procedures are required to start the interpretation of the results. Please refer to 'Interpretation of the Results' on Pages 17–25 for detailed information on the use of the Moda-RA software for microsatellite shift variation analysis.

Materials Provided

Kit contents

Table 3 summarizes the contents of the Modaplex MSI Analysis Kit and indicates the storage conditions.

Table 3: Content of the MSI Analysis Kit

Component	Ingredients	Vials	Vol./Vial (µL)	Cap Color	Storage
Primer Mix MSI	a) FAM or TYE -labelled and unlabelled primer for MSI targets 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	1	15	Orange	-15 °C to -25 °C
Positive Control MSI	a) Control template b) Tris-EDTA buffer	1	65	White	-15 °C to -25 °C
Nuclease-Free Water	a) Nuclease-free water	1	1,500	Light blue	-15 °C to -25 °C

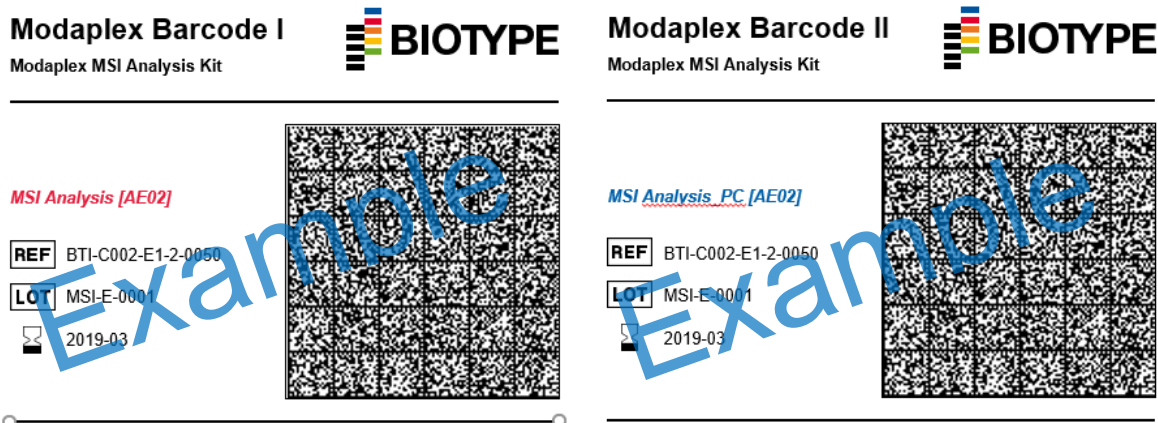
Package Inserts

The Modaplex MSI Analysis Kit is delivered with two packaging inserts—the Modaplex Barcodes and the IFU Download Instruction. Each kit must be used with the packaging inserts supplied therein. It 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 MSI analysis. Both packaging inserts are described in the following subsections.

Modaplex Barcodes

Two barcodes are provided for the detection and quality assessment of sample and control wells. Prior to the first use of a new MSI Analysis Kit, both barcodes must be scanned. On scanning the barcodes, MSI-related assay specifications such as assay ID, 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: Modaplex Barcode I and Barcode II



Modaplex Barcode I contains all MSI kit-related information required for the fragment analysis of MSI.

Modaplex Barcode II contains all information required for the assessment of the PC validity. The assessment of the PC validity differs from the detection of MSI in sample wells. Microsatellite markers and internal control targets must be called on constant fragment length positions in the PC well. In contrast, the sample well algorithm must allow for a flexible assignment of microsatellites, which is the consequence of microsatellite allele variability across ethnicities. In addition, a further size shift is introduced in case of MSI.

To ensure full traceability of kit lot numbers and expiration dates, the both assay-specific Modaplex barcodes need to be scanned prior to the first use of a Modaplex MSI Analysis Kit. With each new lot of the assay, the newly provided barcodes need to be scanned, which result in new assay definitions on the Modaplex instrument, including the new lot numbers and the expiration dates.

Note: In terms of traceability, the assay specifications are present in the Mode-RA report once the barcodes have been scanned and a Modaplex run has been performed with the new MSI 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 MSI IFU is not physically a part of the Modaplex MSI Analysis Kit. However, to guarantee that the kit is used according to the dedicated IFU, the MSI assay comes with a packaging insert 'Modaplex IFU Download'. It gives guidance about how to download the latest IFU version. Therefore, 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 MSI webpage using the link given on the Modaplex IFU download packaging insert:

2. **Click** on the respective IFU link in the MSI download section.

3. **Enter** the IFU Code in the respective field.
The IFU Code is present on the packaging insert 'Modaplex IFU Download'.

Note: If the laboratory environment does not give access to the internet, please contact biotype's support 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 MSI Analysis Kit.

Reagents and Consumables

- Modaplex Calibrator Kit 2 (Biotype)
- DNA extraction kit 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 (various suppliers)
- Sterile microcentrifuge tubes (various suppliers)
- TE Buffer, pH 8.0 (ThermoFisher Cat#: 10006044)
- PCR Microplates 96 (Biotype)
- Mineral Oil (SIGMA)
- Aluminium Sealing Film (Biotype)
- Modaplex CE 48 (Cartridge) (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 (various suppliers)
- Bench-top centrifuge with rotor for 2ml reaction tubes (various suppliers)
- Bench-top centrifuge with plate adaptor (various suppliers)
- Bench-top vortex (various 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 MSI Analysis Kit components, please consult the corresponding safety data sheets (SDS), which are available upon request. For the safety information of the components 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:

- 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.

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 MSI Analysis Kit are formulated specifically for use with the stated test. All the reagents supplied in the kit are intended to be used solely with the other reagents in the same Modaplex MSI Analysis Kit. The reagents in the kit must not be substituted if optimal performance is to be maintained.

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

Reagent Storage and Handling

The Modaplex MSI Analysis Kit is shipped on dry ice. Upon receipt, the assay should immediately be stored at $-15\text{ }^{\circ}\text{C}$ to $-25\text{ }^{\circ}\text{C}$ in a constant-temperature freezer. The fluorescent-labelled 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 diluted optimally and no further treatment is necessary.

Note: Upon receipt, please check the Modaplex MSI Analysis Kit immediately and contact Biotype 's technical service if:

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

Procedure

The Modaplex MSI Analysis Kit has been verified with DNA samples extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue.

DNA purification

It is recommended that DNA purified with the QIAasympphony DSP DNA Mini Kit (Cat# 937236) and QIAamp DNA FFPE Tissue Kit (QIAGEN, Cat#: 56404) is used. DNA purification should be carried out from macrodissected FFPE tissue according to the supplier's instructions.

Note: The Modaplex MSI Analysis Kit is designed for use with short PCR products. However, 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). Both the Qubit™ dsDNA HS Assay (Cat#Q32851) and the Qubit™ ds DNA BR Assay (Cat#Q32850) can be used to measure the DNA concentration after isolation. The DNA concentration of the sample should ideally be 0.4 ng/μl to perform the Modaplex MSI Analysis Kit at the optimal input range of 2 ng. In some cases, the input DNA amount has to be varied within the input range to adjust for variations in DNA yield and quality due to differences in samples. The kit can be performed using 5μl sample DNA with a concentration of 0.1–1 ng/μl (total DNA of 0,5 - 5 ng).

It is not advised to use less than 0.1 ng/μl. An insufficient amount of DNA could lead to low PCR yields and the peak heights may fall below the target-specific detection limits. Excessive amounts of DNA >20 ng may lead to peak heights with a disturbed migration size resulting in incorrect assignments of calibrators, control amplicons, and MSI targets.

Note: If sample material other than the indicated CRC FFPE tissue is used with the Modaplex MSI Analysis Kit a verification of the input DNA amount is recommended.

Protocol: Preparing the Modaplex run for MSI Analysis

This protocol is intended for the preparation of the Modaplex instrument, prior to the set-up of the Modaplex MSI 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 on the cartridge, minus the total number of PCR reactions to be performed.

3. Scan both MSI assay definitions into the Modaplex instrument.

Scan the two Modaplex barcodes provided with the Modaplex MSI 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 MSI Analysis Kit is used.

4. Confirm the Modaplex settings for the MSI Modaplex run.

Before setting up the Modaplex MSI 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
- Adequate quantity of the consumables

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**:

- Tumor tissue (= sample): *sample name_s*
- Normal adjacent tissue (= control): *sample name_c*
- Positive control: *assay name_PC*
- Negative control: *assay name_NC*

Note: It is required to use the same name for both the tumor tissue and normal adjacent tissue from the same patient before the underscore. This is a prerequisite for the Moda-RA Software to be able to align the electropherograms of the two samples automatically.

Note: The sample name can be any unique name, number, or code that can identify a sample. Sample names must be unique for each pair. If there are duplicates on one plate, the naming must differ between them. . 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 MSI Analysis Modaplex run

This protocol is for the preparation of reagents in the Modaplex MSI Analysis Kit and the PCR plate for the Modaplex run.

1. Remove and thaw the following components from the Modaplex MSI Analysis Kit:

- Modaplex Buffer 3 (black cap)
- Modaplex Enhancer 50 (natural cap)
- Primer Mix MSI (red cap)
- Nuclease Free Water (light-blue cap)
- Positive Control MSI (white cap)
- Modaplex Polymerase T (orange cap)

Note: The Modaplex Polymerase T is temperature-sensitive. Keep the enzyme at $-20\text{ }^{\circ}\text{C}\pm 5\text{ }^{\circ}\text{C}$ 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.

4. Preparing the MSI 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 each reagent.

Table 4: MSI 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
Modaplex Buffer 3	12.5 μL	25.0 μL	37.5 μL	50.0 μL	62.5 μL	125.0 μL
Primer Mix MSI	2.5 μL	5.0 μL	7.5 μL	10.0 μL	12.5 μL	25.0 μL
Nuclease Free Water	2.3 μL	4.6 μL	6.9 μL	9.2 μL	11.5 μL	23 μL
Modaplex Enhancer 50	2.0 μL	4.0 μL	6.0 μl	8.0 μl	10.0 μl	20 μl
Modaplex Polymerase T (5.0 U/ μL)	0.2 μL	0.4 μl	0.6 μl	0.8 μl	1.0 μl	2 μl
Calibrator 2	0.5 μL	1.00 μL	1.5 μL	2.0 μL	2.5 μL	5.0 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 MSI Master Mix to the designated wells in the PCR plate.

7. Add 5 μL each of the following:

- Extracted DNA (template) to the corresponding sample well(s)
- Nuclease Free Water to the NC well
- PC MSI to the positive control well

Note: Thus, the total volume of the PCR reaction is 25 μL . 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, which are not being used for a sample well, NC or PC.
 - Prepare a 1:10 dilution of the 10x injection buffer in water.
 - Add 25 μ L 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 21 samples (tumor: tumoral tissue; native: normal adjacent tissue)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 01 Tumor MMx	Sample 01 Native MMx	Sample 09 Tumor MMx	Sample 09 Native MMx	Sample 17 Tumor MMx	Sample 17 Native MMx						
B	Sample 02 Tumor MMx	Sample 02 Native MMx	Sample 10 Tumor MMx	Sample 10 Native MMx	Sample 18 Tumor MMx	Sample 18 Native MMx						
C	Sample 03 Tumor MMx	Sample 03 Native MMx	Sample 11 Tumor MMx	Sample 11 Native MMx	Sample 19 Tumor MMx	Sample 19 Native MMx						
D	Sample 04 Tumor MMx	Sample 04 Native MMx	Sample 12 Tumor MMx	Sample 12 Native MMx	Sample 20 Tumor MMx	Sample 20 Native MMx						
E	Sample 05 Tumor MMx	Sample 05 Native MMx	Sample 13 Tumor MMx	Sample 13 Native MMx	Sample 21 Tumor MMx	Sample 21 Native MMx						
F	Sample 06 Tumor MMx	Sample 06 Native MMx	Sample 14 Tumor MMx	Sample 14 Native MMx	MSI_PC MMx	MSI_NC MMx						
G	Sample 07 Tumor MMx	Sample 07 Native MMx	Sample 15 Tumor MMx	Sample 15 Native MMx	Empty 1x IB	Empty 1x IB						
H	Sample 08 Tumor MMx	Sample 08 Native MMx	Sample 16 Tumor MMx	Sample 16 Native MMx	Empty 1x IB	Empty 1x IB						

9. **Seal** the PCR plate with aluminium 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 aluminium sealing film. Spin the PCR plate and CE plate in a table-top centrifuge.

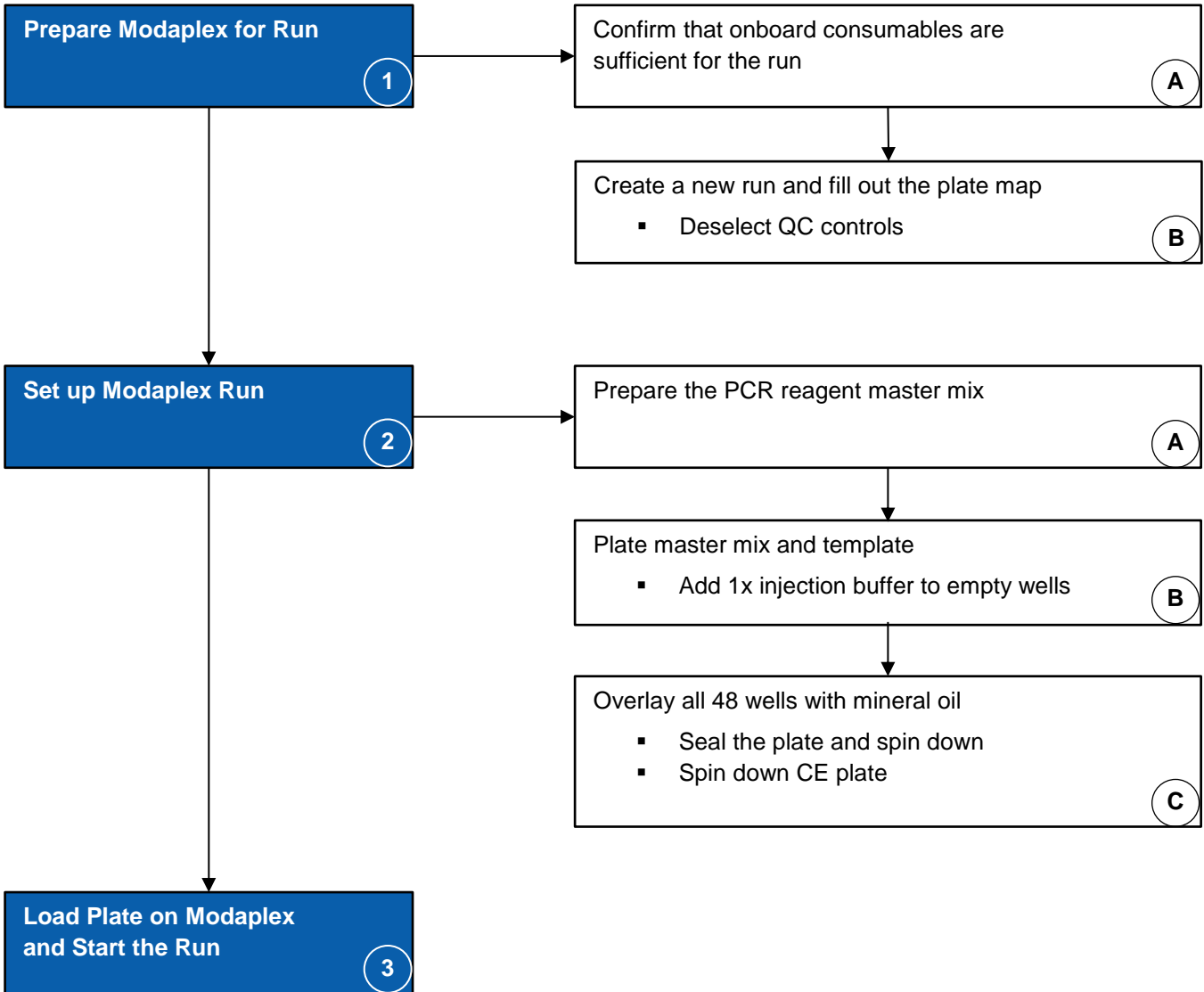


The plate cover seals must be **removed** from the PCR plate and the CE plate before they are placed on the Modaplex instrument.

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 aluminium sealing film before disposing of the plates. Decontaminate the hold-down plate.

Note: Refer to the Modaplex System IFU for further instructions

Procedure—Flow Chart



Interpretation of the Results

After the completion of the Modaplex run, the MSI data must be analysed by the following procedure:

1. Data transfer to the Moda-RA software
2. Data analysis
3. MSI report creation

Data transfer to the Moda-RA software

The following section describes how the MSI 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 related to each Modaplex run, containing all the run-related data. The folder is generated automatically and named with a consecutive number like 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 the folder where your run folder is saved and click on it.

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

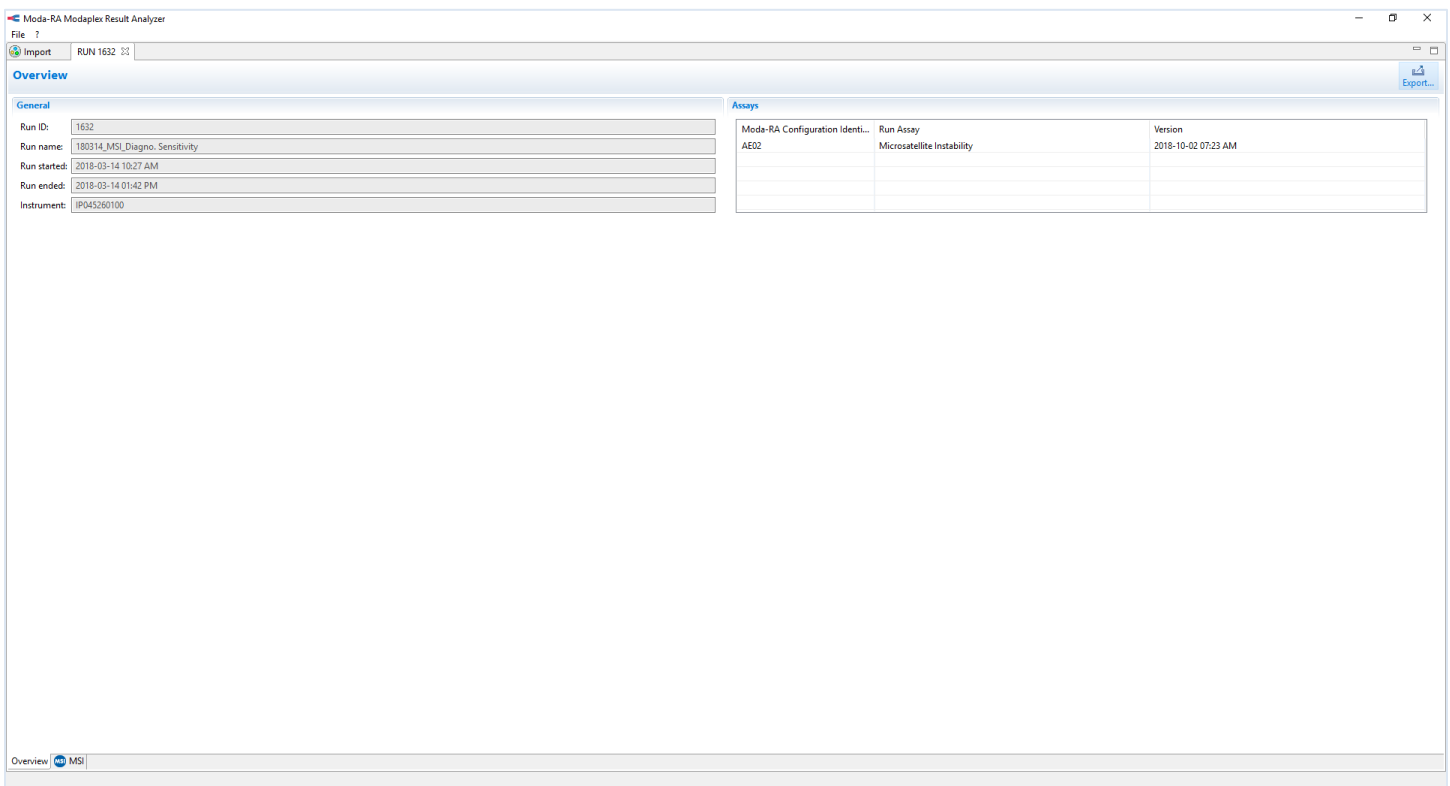
The following section describes how the Moda-RA software is used for the analysis of the MSI data and how the results are displayed. This section also contains detailed information about the result interpretation of the control and the sample wells, including the comments and recommendations for all 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 window "Overview" 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. Please refer to Figure 4 for an example.

Figure 4: Example of the 'Run Overview' window



B. Plate summary and general display of the analyzed data

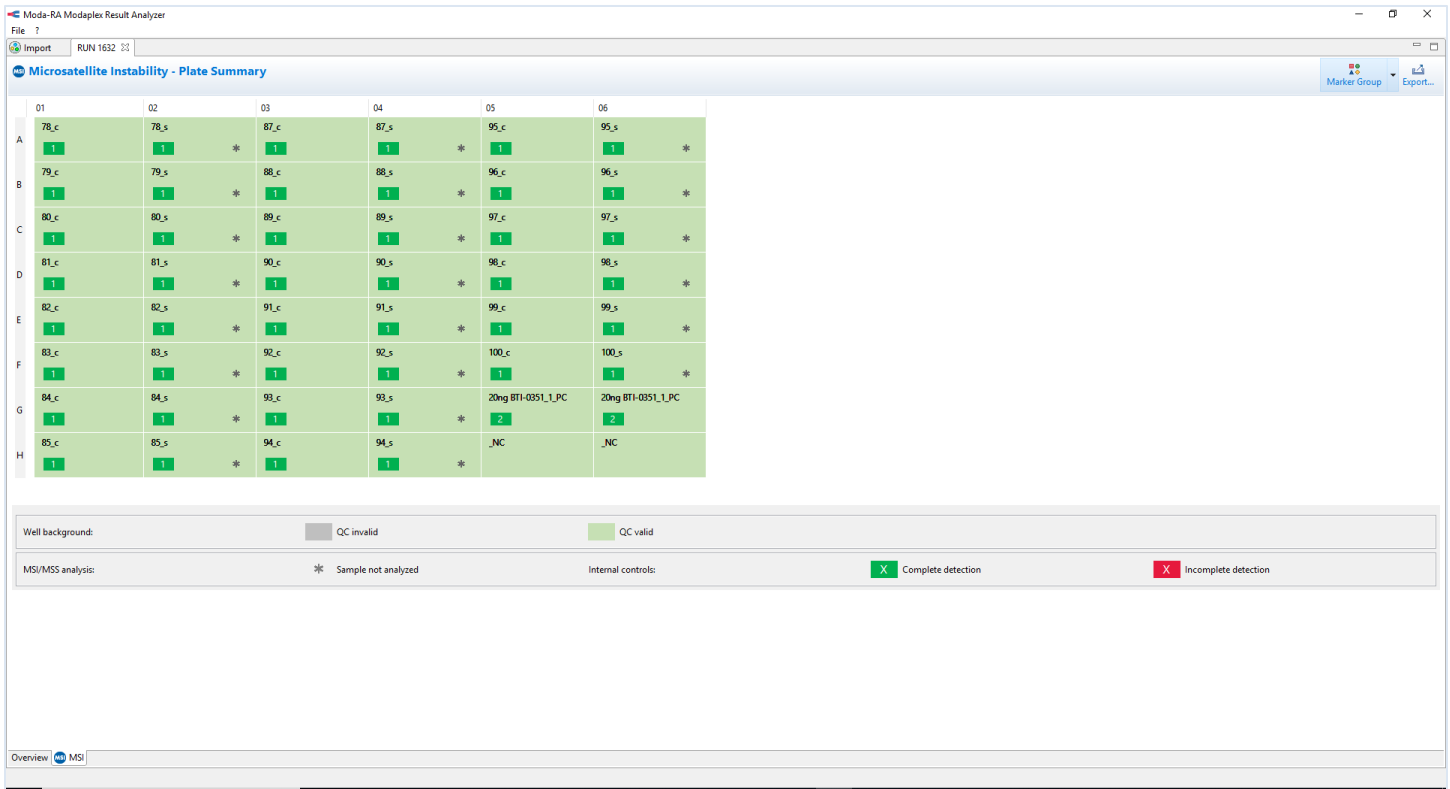
The 'Microsatellite Instability—Plate Summary' window opens when selecting 'MSI' in the 'Run Overview' window. It displays all controls and samples present on the plate that has been imported into the Moda-RA software.

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, regardless of allelic heterogeneity

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

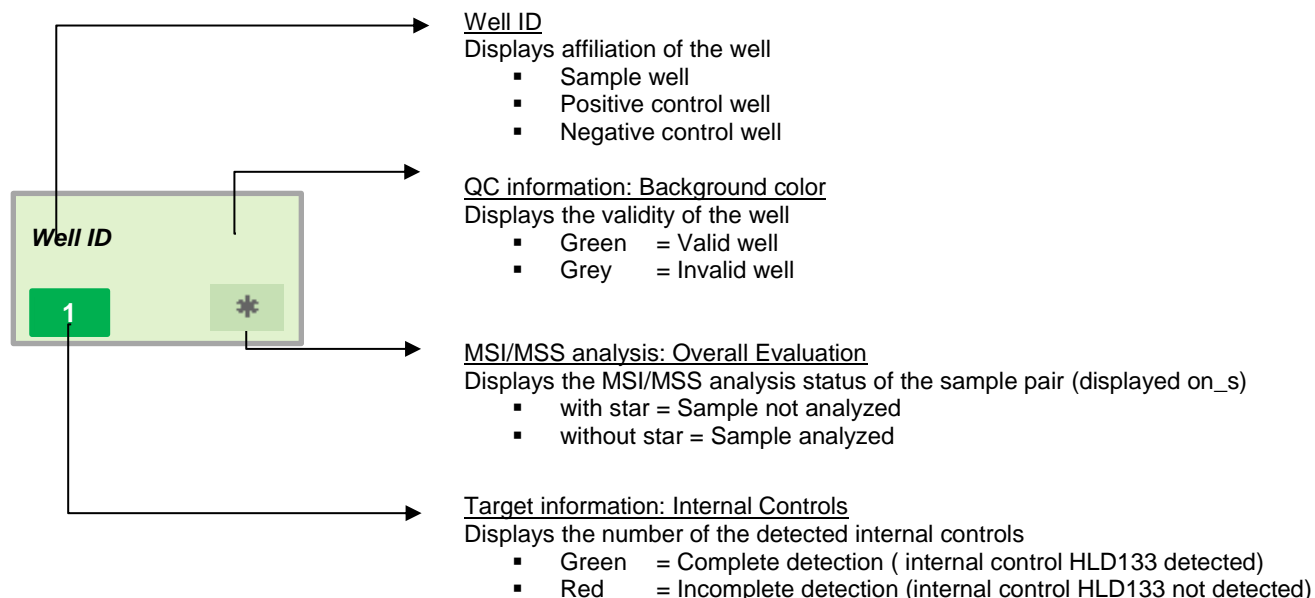
Figure 5: Illustration of the plate summary



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: For MSI data analysis, the sample wells have to be marked according to the following code:

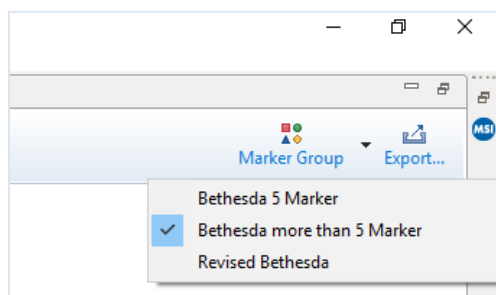
Tumor tissue (= sample): *sample name_s*

Normal adjacent tissue (= control): *sample name_c*

Note: Detailed information on the interpretation of the control and the sample wells is given in Tables 5–8.

The 'Microsatellite Instability—Plate Summary' displays a button labelled '**Marker Group**' above the plate map. By clicking onto this button, the marker group of your interest can be selected. Each marker group displays an individual MSI-H assessment option according to the Bethesda and revised Bethesda guidelines.

Figure 7: Illustration of the Toolbar 'Marker Group'



C. Quality control: Analysis of the NC and PC

Each Modaplex MSI run should include the run controls NC and PC. A Modaplex run is valid if the PC and the NC runs 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 with the validity of the sample wells. A sample well can be valid even when there are invalid run controls. In such a case, the reason behind the invalid controls should be investigated. Biotype should be contacted for technical assistance, if required.

1. Negative Control (NC)

The NC is reported as valid if no internal control (HLD marker) is detected. It is reported as invalid if at least one internal control is detected. Please review the Biotype troubleshooting guide in case of a failed NC.

Note: The area between Calibrators 1 and 2 can contain additional peaks. Those peaks represent specific technical-issue-related AB. They do not influence the validity of the NC.



Note: For more detailed information, please refer to Tables 5 and 6.

2. Positive Control MSI (PC)

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




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

Table 5. Moda-RA: Valid and invalid run controls

Background color of control wells	Description	Criteria
	<ul style="list-style-type: none"> Valid Modaplex run for PC and NC wells 	<ul style="list-style-type: none"> At least 2 out of 3 calibrator peaks detected in NC or PC (calibrator peak 1 must be present) All markers detected in PC Both HLD131 and HLD133 are detected in PC No markers detected in NC No internal controls detected in NC
	<ul style="list-style-type: none"> Invalid Modaplex run for PC and NC wells 	<ul style="list-style-type: none"> Calibrator 1 not detected in NC or PC Calibrator 2 and 3 are not detected Less than 8 markers detected in PC Less than two internal controls detected in PC Detection of a marker in NC Detection of any internal control 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: The information for the run control

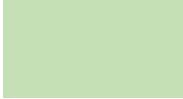

Color and number code for the positive control	Description	Criteria
	<ul style="list-style-type: none"> Complete detection of internal control 	<ul style="list-style-type: none"> Displays the two detected internal controls - HLD133 and HLD133
	<ul style="list-style-type: none"> Incomplete detection of internal controls 	<ul style="list-style-type: none"> Displays one detected internal control - HDL131 or HDL133
	<ul style="list-style-type: none"> Incomplete detection of internal control 	<ul style="list-style-type: none"> Internal control HLD133 not detected

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

D. Result Interpretation: Analysis of the sample wells



The 'Microsatellite Instability Panel Summary' 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 7 and 8 describe the color coding in detail. The wells are characterized according to the number of calibrators and the number of internal controls.

Table 7. Moda-RA software: The QC information for the sample wells

Background Color of sample wells	Description	Criteria
	<ul style="list-style-type: none"> Valid sample well 	<ul style="list-style-type: none"> At least 2 out of 3 calibrator peaks detected (calibrator peak 1 must be present) Internal control HLD133 detected in PC
	<ul style="list-style-type: none"> Invalid sample well 	<ul style="list-style-type: none"> Calibrator 1 not detected Calibrators 2 and 3 not detected Internal control HLD133 not detected

Note: In some cases, the largest D2S123 alleles can't be assessed reliably due to the ABs. In patients, homozygous for these alleles, D2S123 instability might not be detectable. In these cases, we recommend to include all microsatellite markers to assess the MSI.

Table 7. Moda-RA software: The information for the run sample wells

Color and number code for the sample wells	Description	Criteria
	<ul style="list-style-type: none"> Complete detection of internal control 	<ul style="list-style-type: none"> Displays the detected internal control HLD133
	<ul style="list-style-type: none"> Incomplete detection of internal controls 	<ul style="list-style-type: none"> Internal control HLD133 not detected

To simplify the analysis of the sample pairs, the well of the tumor samples—labelled with `_s` is shown with an asterisk (*). This label vanishes when the tumor sample is evaluated.

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 electropherograms.

A tab opens on the right for each well. The marker panel of choice is displayed on the top. Each marker can be evaluated. If there is a corresponding sample -either `_s` or `_c`, named identically before the underscore, you can automatically see the overlay of both signals in the electropherograms.

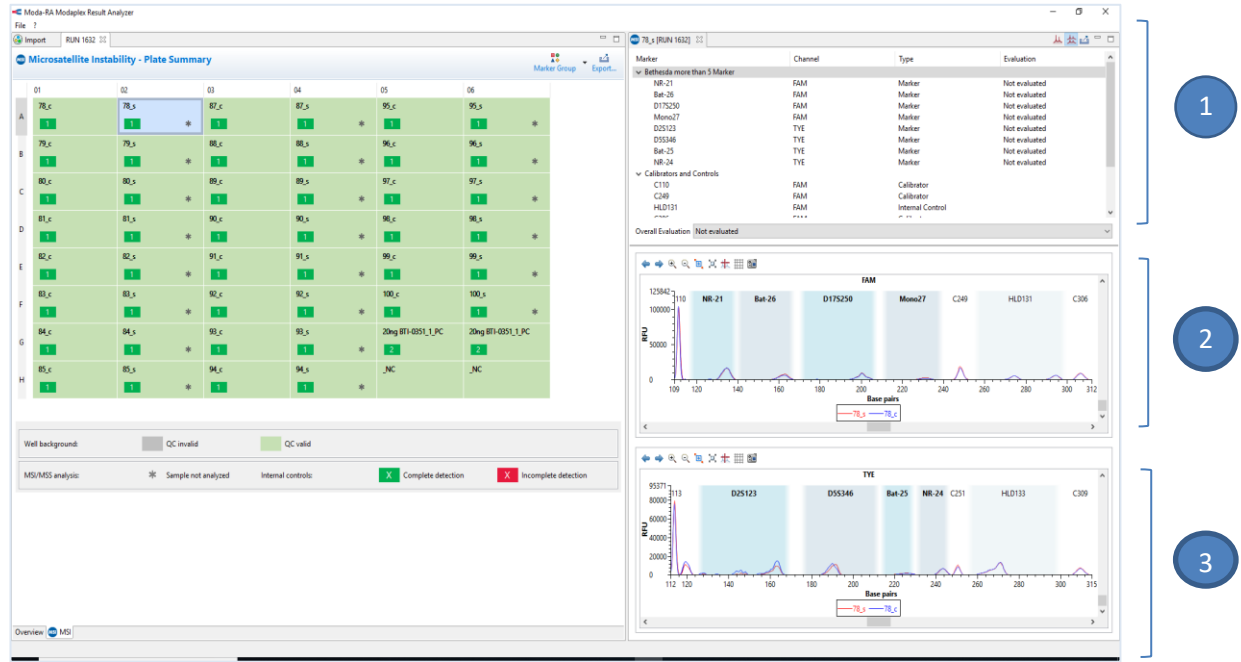
Note: If the naming of the sample pair is not identical you can individually overly the samples by using the following toolbar icon: This will open a pop-up window display all available well. Please select the well for overlay.



3. By clicking on the marker in the marker panel (Section #1, Figure 8), an automatic zoom into this marker occurs. You can start evaluating each marker immediately.

4. Below the marker panel and the two electropherograms are displayed. The EPG of the FAM channel is displayed in Section #2 and that of the TYE channel in Section #3. The range of each marker is clearly shown. You can individually zoom into each one.

Figure 8: Data analysis of sample wells (the blue numbers show the different sections).



By default, the red line represents the signal of the sample well (_s) and the blue line the signal of the control well (_c). The unit of the Y-axis of the coordinate system is RFU, whereas the shared X-axis displays 'base pairs' (bp).

Note: Technical-issue-related AB occur. The bands do not affect the data MSI analysis and are therefore negligible. They are located at the following positions of the Y-axis in the FAM channel and TYE channel: 273 bp (FAM), 118.4 bp (TYE), 161.5 bp (TYE), and 143 bp (TYE).

5. Various toolbar icons (see Table 8) are available for different optical presentations of the electropherograms in Sections #4 and #5 (see Figure 9).

Note: A full screen version of the electropherogram is available as well.

Figure 9: Moda-RA software: Presentation of electropherograms.



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Table 8. Moda-RA software: The toolbar icons of electropherograms.

Icon	Description
	<ul style="list-style-type: none"> With the Arrow icons, you can switch between the previous marker and the next marker.
	<ul style="list-style-type: none"> Use the buttons "Zoom In" and "Zoom Out" in the electropherogram toolbar.
	<ul style="list-style-type: none"> Every part of a coordinate system can be magnified with dynamic zoom. To this end, click with the left mouse button into the representation and draw a frame to the right while keeping the mouse button pressed. Upon releasing the button, the selected area of the coordinate system is magnified. Repeat this procedure as often as you like. To zoom out, press the left mouse button and keep it pressed while drawing the mouse to the left.
	<ul style="list-style-type: none"> Full Zoom out brings you back to the complete representation of the EPG.
	<ul style="list-style-type: none"> Click Show/Hide Cursor to activate cursor. The lines of the cursor extend to the end of the coordinate system. If several related coordinate systems are depicted one below the other, the vertical cursor is shown in the other coordinate systems as well.
	<ul style="list-style-type: none"> A grid is displayed in the EPGs.
	<ul style="list-style-type: none"> Take a snapshot and save as PNG file.
	<ul style="list-style-type: none"> Maximize electropherogram (FAM and TYE channels).
	<ul style="list-style-type: none"> Compare electropherograms (sample and control wells).
	<ul style="list-style-type: none"> Export: create a printable report for documentation or export of data.

6. **Select** 'Stable', 'Unstable', or 'Uncertain' on the evaluation field in Section #1 after the analysis of electropherograms in Sections #2 and #3.
7. **Repeat** the steps for each marker.
8. **Select** the 'Overall Evaluation' field. Click with the left mouse and select 'MSI-High', 'MSI-Low' or 'MSS stable'. The star on the sample well disappears now.

Note: Do not close the well tabs before creating a printable report; otherwise, the evaluation will be lost.

Creating the MSI report

This protocol is for the preparation of a report after the MSI data has been analyzed and classified as valid. The report contains the following information:

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

For creating the MSI 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** a 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.

For creating the MSI report and exporting the report as a *.csv 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** *Export to files* and then select *Next*.
Now you can choose from various csv-format options as well as the sylk-format.
3. **Choose** a folder where you want to save your file.
4. **Select** *Finish*.
The file is created automatically
Note: The document will be automatically named '*Microsatellite Instability*' and should be renamed after saving.

Performance Characteristics

Table 8. Performance characteristics of the Modaplex MSI Analysis Kit

Assay Performance	Result/Description
Primer Specificity	A basic local alignment search tool (BLAST) analysis is performed. The primer of the Modaplex MSI Analysis Kit only amplifies eight targets (Bat-25, Bat-26, NR-21, NR-24, Mono27, D2S123, D5S346, D17S250) and two internal control targets (HLD131 and HLD133).
Sample Material	Human gDNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue.
Input Range	DNA input: 0,5 – 5ng
	Optimum: 2ng

Troubleshooting Guide

The troubleshooting guide may be helpful for solving any problems that may arise. Also, see the Modaplex System User Manual for Instrument and Software Troubleshooting Guidance. The scientists at Biotype 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)	<ul style="list-style-type: none">• 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.• Check the maintenance interval for all used devices (e.g. pipettes)• 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)	<ul style="list-style-type: none">• Incomplete detection of targets, calibrators and/or internal control. 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.• Check the maintenance interval for all used devices (e.g. pipettes)• 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.
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	<ul style="list-style-type: none">• 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 MSI Analysis Kit is past the expiration date	<ul style="list-style-type: none">• 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.
Incorrect overlay of peaks.	<ul style="list-style-type: none">• Calibrators have failed or assigned incorrectly. Please contact the technical support.
Targets only present in one channel	<ul style="list-style-type: none">• Review all the run controls and the sample wells in the Moda-RA software. If no well shows fluorescence in one channel, contact Biotype.
Peaks show unusual forms: CE quality issue - Capillary fails before or during run	<ul style="list-style-type: none">• For support, please contact Biotype.• The input DNA amount may be too high. Repeat the Modaplex run with a lower DNA amount.

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 MSI Analysis Kit IFU is required. The dilution of the reagents, other than as described in this IFU, is not recommended and will result in a loss of performance.

It is important that the amount and quality of the DNA in the sample are assessed prior to performing a sample analysis using the Modaplex MSI 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 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 be liable for any indirect, special, or consequential damages.

References

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Symbols

The following symbols may appear on the packaging and labelling:



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.

Contact Information

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Ordering Information

Product	Contents	Cat. no.
Modaplex MSI Analysis Kit	For 50 reactions: Primer Mix MSI, PCR Buffer 3, Modaplex Enhancer 50, Modaplex Polymerase T, Positive Control MSI, Nuclease-Free Water	BTI-C002-E1-2-0050
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 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
Aluminium Sealing Film	1 Box of 100 Aluminium Sealing Film	00-14X04-0100

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