

Plentiplex™ MSI

Made by PentaBase

READY-TO-USE QUICK GUIDE

Plentiplex™ MSI for Detection of Microsatellite Instability in Cancer patients



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1. INTRODUCTION

This quick guide is meant for experienced users. Please review the full “Instructions for use” carefully before using the kit. The PlentiPlex® MSI assays offer three different multiplexed MSI assays for the length analysis of five microsatellite loci. The mono- and dinucleotide panel recommended by the Bethesda guidelines (PlentiPlex™ MSI Bethesda) and two Pentaplex mononucleotide panels, enabling evaluation of MMR status on DNA sequencer instruments.

2. CONTENT

PlentiPlex™ MSI assays are supplied as ready-to-use assays in either 12 or 60 reactions in pre-aliquoted 0.2 ml PCR strips or tubes suitable for most standard 96-well PCR instruments. All reagents needed for the PCR amplification of extracted genomic DNA are pre-loaded into the strips or tubes.

3. STORAGE AND STABILITY

The unopened product is stable at -20°C for a minimum of 9 months, but no longer than the expiry date.

Important: Keep frozen until use and thaw at room temperature. Avoid repeated freeze/thaw cycles.

4. DNA EXTRACTION

- Use FFPE, fresh frozen or any other suitable biopsy material
- Genomic DNA (gDNA) can be extracted using any valid gDNA extraction kit
- Follow the instructions for gDNA extraction recommended by the kit supplier
- Determine the quantity and quality of gDNA prior to real-time PCR. Do not use gDNA of a low quality
- Use 5-200 ng gDNA per reaction/tube

5. KIT PREPARATION

Thaw one strip or tube per sample per analysis and spin down before removing lids gently

6. SETUP

When using the Bethesda panel, it is mandatory to include a germline (non-tumor) patient gDNA sample for comparison with the corresponding patient tumor sample. Analysis of patient tumor samples with the Classic and PentaBase panel can be carried out using either a patient-matched or non-matched germline DNA sample as reference.

- Add 5 µL extracted patient DNA (1-40 ng/µL) per tube
- Gently mix with a pipette, seal vial and spin down
- Place strips or tubes in a PCR instrument and run the protocol shown in Table 1

Table 1. PlentiPlex® MSI PCR protocol

| Protocol | Temperature | Time | Number of cycles |
|----------|-------------|----------|------------------|
| Hold | 94°C | 600 sec. | 1 |
| Cycling | 94°C | 50 sec. | 10 |
| | 55°C | 50 sec. | |
| | 72 °C | 50 sec. | |

| | | | |
|---------|-------|---------|----|
| Cycling | 89°C | 30 sec | 25 |
| | 55°C | 30 sec. | |
| | 72 °C | 30 sec. | |
| Hold | 72 °C | 10 min. | 1 |
| Hold | 10 °C | ∞ | 1 |

After PCR, dilute PCR amplicons 1:10 with DNase free water

- Mix 1 µL of diluted DNA template with 9 µL of formamide and 0.25 µL of size standard DNA ladder in plasticware compatible with DNA fragment/sequence analyzer configurations
- Place in a heating block at 95°C for 120 secs
- Place immediately on ice bath at 4°C for at least 10 min
- Evaluate amplicon size applying standard protocols on DNA sequence analyzer

7. NOTES

- All test components should be stored as described in the “Instructions for use” (storage section)
- Do not mix reagents from different lots
- Always spin down before opening the lids

8. DATA ANALYSIS

Micro-satellite loci are evaluated individually by comparing the length of amplicons obtained with the reference gDNA to those observed when applying the patient’s tumor-derived gDNA. Both shorter and longer micro-satellite amplicons are indicative of instability. When using the Bethesda panel, and the Classic and PentaBase panel with patient germline (non-tumor) gDNA as reference, with five micro-satellites analyzed, patients can be categorized as MSS (0 markers exhibiting instability), MSI-L (1 marker being unstable) or MSI-H (2 or more loci being unstable). When using the Classic and PentaBase panel with non-matched wild type gDNA as reference, patients can be categorized as MSS (0 markers exhibiting instability) or MSI-H (3 or more loci being unstable). When only 1 or 2 of the 5 loci exhibit micro-satellite length alteration(s) it is recommended to rerun the sample preferably with inclusion of patient-matched germline (non-tumor) gDNA as reference. If a rerun with non-matched wild type gDNA as reference produces the same results, the sample is considered MSS.

Typical size ranges are described in Tables 2-4.

Table 2: MSI Bethesda Panel loci identification

| Marker | Detected in channel | Label | Typical size range (bp) |
|---------|---------------------|---------------|-------------------------|
| BAT25 | Blue | PentaGreen | 121-127 |
| BAT26 | Red | PentAltRed | 122-128 |
| D2S123 | Blue | PentaGreen | 197-227 |
| D5S346 | Yellow | PentAltOrange | 96-132 |
| D17S250 | Green* | PentAltYellow | 147-169 |

*Risk of crosstalk between green and yellow channel, so that green signal can be observed in yellow channel.

Table 3: MSI Classic Panel loci identification

| Marker | Detected in channel | Label | Average size (bp) |
|--------|---------------------|---------------|-------------------|
| BAT25 | Blue | PentaGreen | 120 |
| BAT26 | Red | PentAltRed | 124 |
| NR-21 | Green* | PentAltYellow | 103 |
| NR-22 | Blue | PentaGreen | 142 |
| NR-24 | Green* | PentAltYellow | 132 |

*Risk of crosstalk between green and yellow channel, so that green signal can be observed in yellow channel.

Table 4: MSI PentaBase Panel loci identification

| Marker | Detected in channel | Label | Average size (bp) |
|---------|---------------------|---------------|-------------------|
| BAT25 | Blue | PentaGreen | 120 |
| BAT26 | Red | PentAltRed | 124 |
| MONO-27 | Red | PentAltRed | 148 |
| NR-22 | Blue | PentaGreen | 142 |
| NR-24 | Green* | PentAltYellow | 132 |

*Risk of crosstalk between green and yellow channel, so that green signal can be observed in yellow channel.

Please refer to the “Instructions for use” for examples of Micro-satellite instability.

9. TROUBLESHOOTING

This short troubleshooting guide may assist in solving most frequent encountered problems that can occur. Please refer to the “Instructions for use” for further troubleshooting.

- If there is low or no signal, try to rerun the PCR and/or re-extract gDNA from the sample
- Extra signals in the yellow channel can be due to cross signal from primers labelled with PentaAltYellow. Please disregard signals in the yellow channel above 140 base pairs.

The full version of the “Instructions for use” can be found at www.pentabase.com.
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