Data Sheet

Jena Bioscience
Building Blocks of Life

HT-MSI⁺ Analysis Kit

Analysis of Microsatellite Instability (MSI) in Tumor Cells by Fluorescent Multiplex PCR

Cat. No.	Amount
PCR-704	100 reactions

For general laboratory use

Shipping: shipped on blue ice
Storage Conditions: store at -20 °C
Additional Storage Conditions: avoid freeze/thaw cycles
Shelf Life: 12 months
Form: liquid

Description

HT-MSI⁺ Kit detects the presence of microsatellite instability (MSI) via fluorescent hexaplex PCR followed by capillary electrophoresis fragment analysis. The kit is designed for analysis of DNA samples from tumor cells derived from fresh (i.e. blood), frozen or from fixed formalin paraffin-embedded tissues (FFPE). The assay analyses a panel of 6 quasi-monomorphic mononucleotides NR-27, NR-21, NR-24, BAT-25, BAT-26 and HPS110. This panel is an expanded optimized version of the standard panel with only five mononucleotides (Goel *et al.* 2010) since it includes the HSP110 microsatellite T17 intron repeat, whose mutation has been highly associated with MSI positive colorectal cancer cells lines (Dorard *et al.*, 2011). The kit also allows MSI analysis in tumors without the need of reference DNA due to the quasi-monomorphic variation range (QMVR) for MSI negative samples already established for each marker (Campanella *et al.*, 2014 and Berardinelli *et al.* 2018). All 6 fragments are amplified in a unique hexaplex PCR reaction that includes a hot start polymerase of high specificity in an advanced buffer formulation designed for samples of various sources. The separation and size analysis of the fluorescent amplified fragments can be carried out in any automated Fragment Analyzer system containing filters in the blue, green, yellow and red regions.

Markers and probes included in the kit

Marker	Dye	Color	QMVR for MSI⁺ (bps)*
NR-21	6-FAM	Blue	101-107
BAT-26			174-180
NR-27	JOE	Green	82-88
BAT-25			142-148
NR-24	Atto550	Yellow	124-129
HSP-110	Atto565	Red	147-150

* QMVR: quasimonomorphic variation range established from the analysis of normal DNA samples considering the mean allele size of each marker with a range ± 3 bps (Goel A, *et al.* 2010; Campanella *et al.* 2014 and Berardinelli *et al.* 2018).

Content

Component	Cap Color	Volume
HT-MSI⁺ Multiplex Master (2x) Hot start polymerase, dNTPs, complete reaction buffer, stabilizer	purple	500 µl
HT-MSI⁺ Primer Mix Hexaplex (10x) Mix of 6 primer pairs	brown	100 µl
HT-MSI⁺ Positive Control PCR Positive control solution for amplification of negative MSI	yellow	10 µl
HT-MSI⁺ Nuclease-free Water	white	400 µl

Not included in the kit

Internal size standard for fragment size calibration; formamide

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Protocol

1. PCR reaction setup

Materials to be supplied by the user:
- Thermal cycler
- Micro centrifuge
- Sterile micro centrifuge tubes from 0.2 ml, 1.5 ml or 96-well plates
- filter tips

NOTE: It is recommended to use a DNA sample of a minimum concentration of 2 ng/µl in a reaction volume of 10 µl following the protocol below. Please note that depending on the quality of the DNA sample, optimization of the final DNA concentration in the reaction might be necessary.

In order to avoid contamination from human genomic DNA into the samples and cross-contamination within the reactions, it is strong recommended to use gloves and sterile filter tips. Prepare the mother reaction mix of all components except template DNA to reduce pipetting errors and setup the reaction in an area separated from the DNA preparation and analysis. Use equipment and supplies dedicated for the amplification setup.

a. Make sure that all the reagents are well mixed prior reaction set up. It is possible to vortex the Mix HT-MSI⁺ for 5-10 seconds to resuspend any possible precipitation
b. On ice, prepare the reaction master mix sufficient for the number of samples being analyzed, considering both positive and negative controls plus one reaction according to the table below:

Number of samples	9
Reaction for PCR positive control	1
Reaction for PCR negative control	1
Total number of reactions plus one (11 + 1)	**12**

Master mix set-up

Components	Volume per 10 µl reaction	Volume for 12 reactions
HT-MSI⁺ Multiplex Master Mix (2x)	5 µl	60 µl
HT-MSI⁺ Primer Mix Hexaplex (10x)	1 µl	12 µl
HT-MSI⁺ Nuclease free water	3 µl	36 µl
Total Volume Reaction Master Mix	**9 µl**	**108 µl**

c. Dispense 9 µl of the mother reaction mix into 0.2 ml PCR tubes or 96-well PCR plate and add 1 µl of the DNA sample (≥ 2 ng/µl) to be analyzed. For the positive and negative PCR controls add in separated tubes or wells 1 µl of the kit HT-MSI⁺ Positive Control PCR and 1 µl of the kit HT-MSI⁺ Nuclease free water respectively.

NOTE: Keep all DNA samples in nuclease free water or TE buffer (10 mM Tris HCl pH 8.0, 0.1 mM EDTA). For buffers of different pH or EDTA concentration above 0.1 mM, the DNA sample volume should not exceed 20% of the final reaction volume. The quality and efficiency of the amplification can be impaired in different pH values, high concentration of MgCl2 or possible inhibitors coming from the DNA sample.

2. PCR Cycling

Steps	Temperature	Duration	Cycles
Initial denaturation	95 °C	15 min	1
Denaturation	95 °C	30 sec	35 - 40
Annealing	55 °C	90 sec	
Extension	72 °C	30 sec	
Final extension	72 °C	40 sec	1
Final	4 °C	∞	1

Keep the PCR reactions at - 20 °C and protect from light

HT-MSI+ Analysis Kit

Analysis of Microsatellite Instability (MSI) in Tumor Cells by Fluorescent Multiplex PCR

3. Reaction setup for fragment analysis

a. Dilute 1 µl of the PCR product in 49 - 99 µl nuclease free water. The dilution level is dependent on the sensitivity of the equipment used
b. Transfer 1 µl of the diluted PCR product to the sequencer microplate
c. Add 8.7 µl of formamide + 0.3 µl of internal size standard
d. NOTE: We recommend to use Hi-Di™ Formamide (Thermo #4311320) and GeneScan™ 600 LIZ™ dye Size Standard v2.0 (Thermo #4408399)
e. Incubate the samples for denaturation at 95 °C for 5 minutes
f. Proceed with the fragment analysis in a capillary electrophoresis (CE) genetic analyzer containing filters in the blue, green, yellow and red regions. Follow the equipment instructions.

4. Data interpretation

The products of the multiplex PCR reaction are separated according to their size by capillary electrophoresis and visualized as electropherogram where every peak represents fluorescently labeled fragments of equal sizes in base pairs. The fluorescent signal is proportional to the number of DNA molecules containing that specific fluorophore. The complete panel comprises a distribution of peaks at specific sizes, where the major allele peak corresponds to the size of one of the 6 mononucleotide markers analyzed. Please note that the presence of one or two major allele peaks is dependent on whether the individual is homozygous (one peak as in panel A, NR-27 marker) or heterozygous (two peaks as in panel A, NR-21 marker) for that marker. Moreover, less intense stutter peaks at 1bp intervals from the major allele peak are also seen due to polymerase slippage during PCR amplification of short tandem repeats.
A MSI-negative DNA sample exhibits microsatellite regions of lengths within the quasimonomorphic variation range QMVR. All 6 mononucleotides analyzed in this kit are quasimonomorphic, so that the normal allele length variation is low and within the determined QMVR of each marker. Alterations due to deletion or insertion of a repeating unit produce an allele of different length so that the fragment analysis shows a marker with a prominent peak outside the QMVR, signalizing instability of this specific microsatellite region. Instability of ≥ 2 markers within a sample panel are classified as MSI-positive (MSI+, panel B). Samples of ≤ 1 altered markers are generally classified as MSI-negative (MSI-, panel A). Nevertheless, it is unlikely that cells with an intact DNA mismatch repair (MMR) will exhibit instability in one of the markers from the panel. If the case, we recommend reanalysis of the sample or an additional analysis approach prior sample characterization in respect to its MSI status.

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HT-MSI+ Analysis Kit

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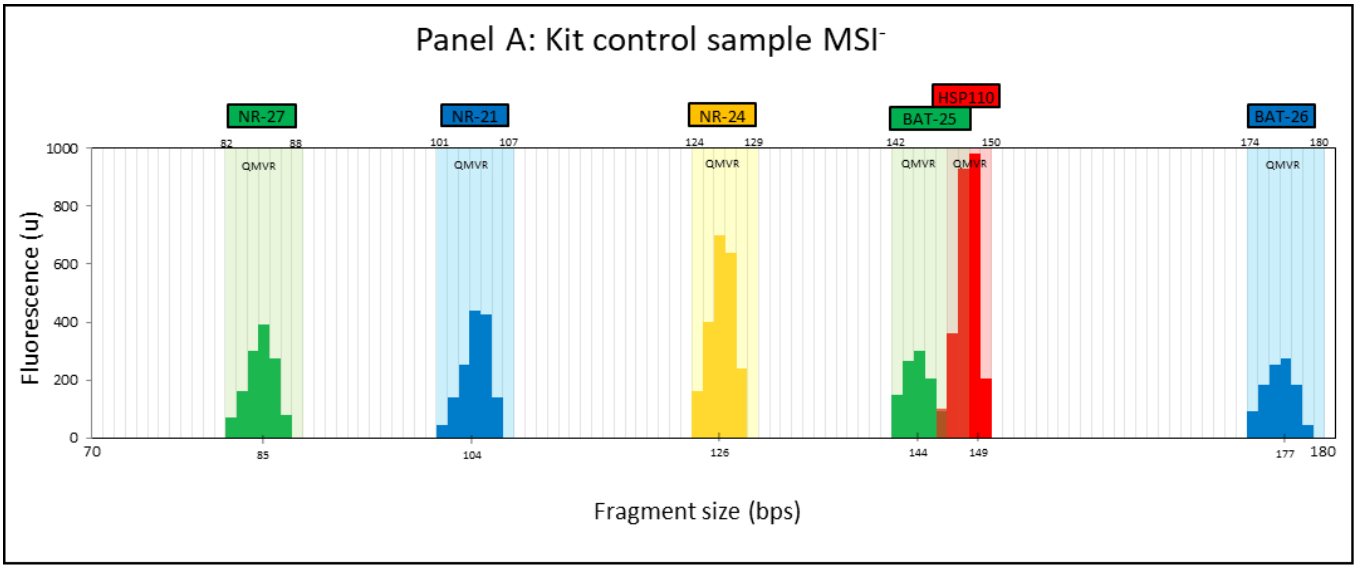


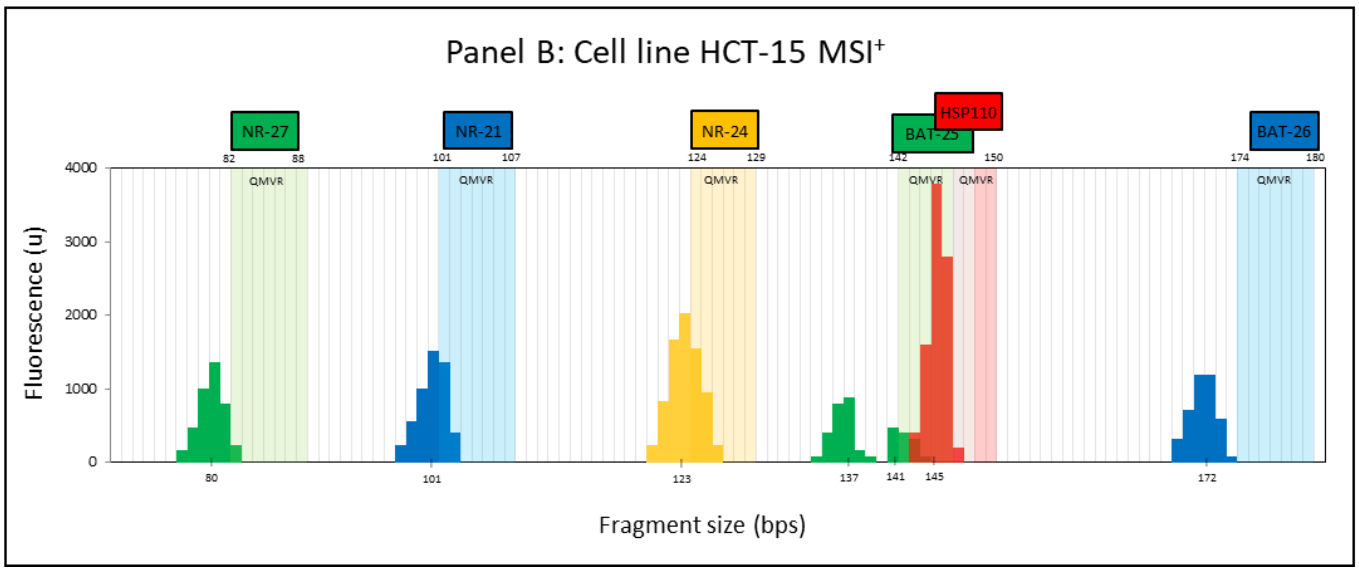
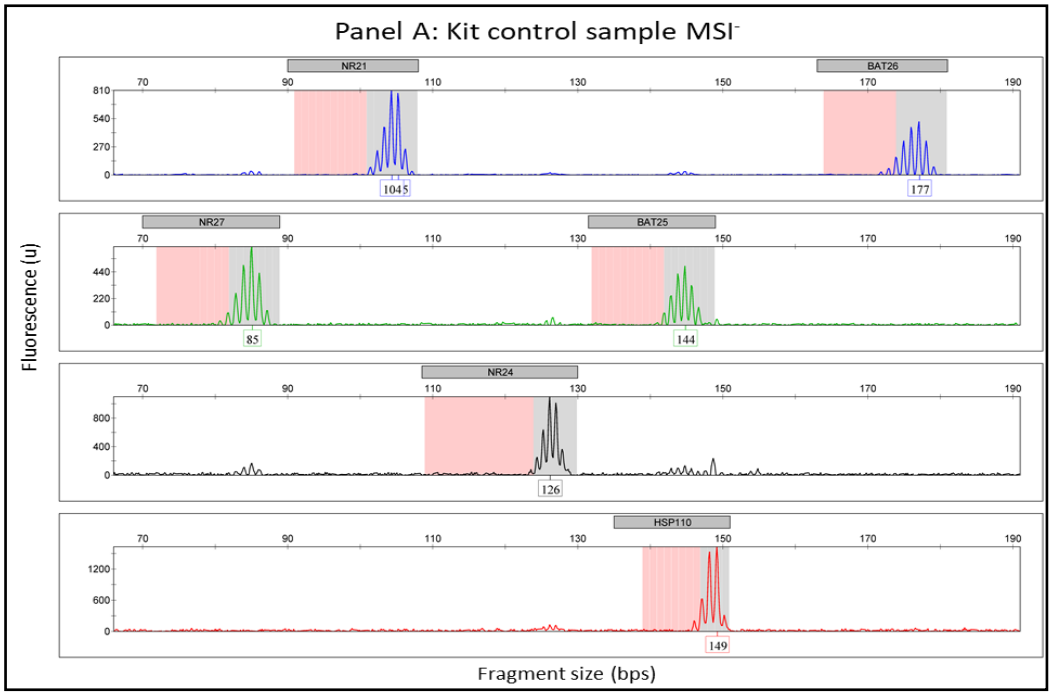


Figure 1: Electropherogram of two different DNA samples showing the size distribtuion of 6 mononucleotide markers NR-27, NR-21, NR-24, BAT-25, BAT-26 and HSP110. The color of the peaks represent the wavelength corresponding to each fluorecent marker. The shaded region shows the quasimonomorphic variation range (QMVR) of each marker, representing no instability within this area. A fragment size outside this area indicates the presence of microsatelite instability for the marker. Panel A: Electropherogram showing the marker size distribution

HT-MSI+ Analysis Kit

Analysis of Microsatellite Instability (MSI) in Tumor Cells by Fluorescent Multiplex PCR

from the control sample included in this kit, characterized as MSI-. Panel B: Electropherogram showing the marker size distribution from a colorectal carcinoma cell line HCT15 where all 6 markers display instability and so sample is characterized as MSI+.



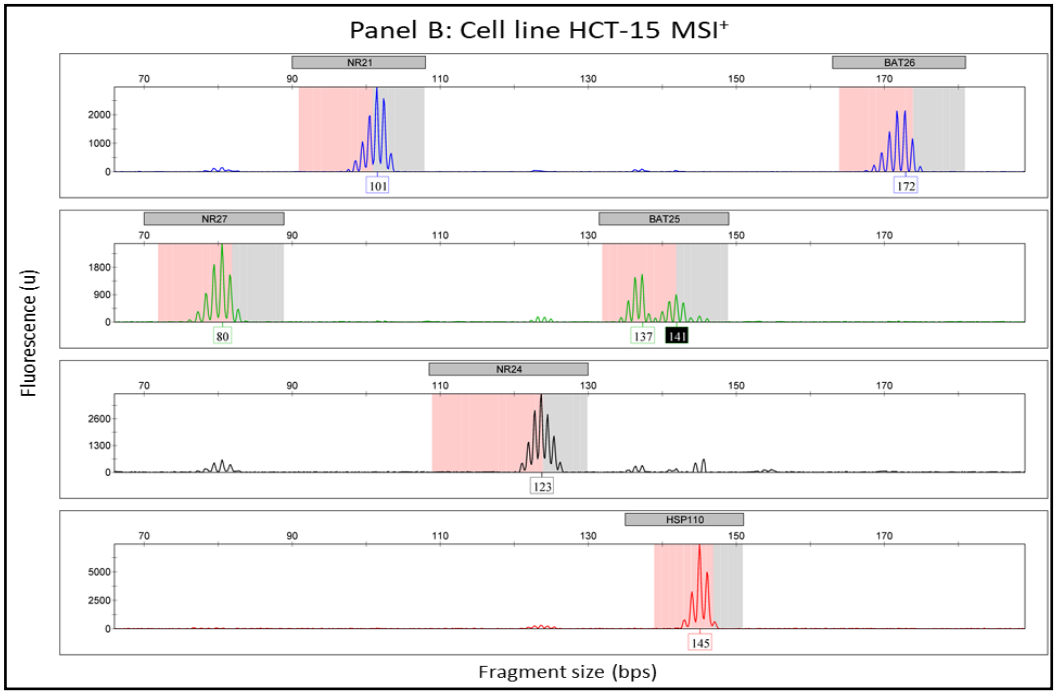


Figure 2: Fragment analysis using the 3500 Genetic Analyzer

HT-MSI+ Analysis Kit

Analysis of Microsatellite Instability (MSI) in Tumor Cells by Fluorescent Multiplex PCR

(Applied Biosystems). Both panels were processed with the software GeneMapper 4.1 (Applied Biosystems) and correspond to the panels A and B in Fig 1. The markers here are though displayed separately according to the wavelength: blue for the NR-21 and BAT-26; green for NR-27 and BAT-25; yellow (electropherogram in black) for NR-24 and red for HSP-110. The shaded areas in gray represent the quasimonomorphic variation range QMVR and red the region that indicates marker instability.

References

Berardinelli *et al.* (2018) Advantage of HSP110 (T17) marker inclusion for microsatellite instability (MSI) detection in colorectal cancer patients. *Oncotarget* **9 (47):**28691.

Campanella *et al.* (2011) Optimization of a pentaplex panel for MSI analysis without control DNA in a Brazilian population: correlation with ancestry markers. *Eur. J. Hum. Genet.* **22 (7):**875.

Goel *et al.* (2010) An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers. *PLoS One.* **5 (3)**

Dorard *et al.* (2011) Expression of a mutant HSP110 sensitizes colorectal cancer cells to chemotherapy and improves disease prognosis. *Nat.. Med.* **17:**1283.