




Certificate of Analysis

SALSA® MS-MLPA® Probemix ME012 MGMT-IDH1-IDH2

Catalogue #	ME012-25R, ME012-50R, ME012-100R	
Product name	Probemix ME012 MGMT-IDH1-IDH2	
LOT	A1-0419	
	25, 50, or 100 reactions.	
Shipping conditions	Dry ice or cooling elements.	
	Store upon arrival between -25°C and -15°C.	
	Expiration date: April 2024, when stored at recommended conditions. This product should not be frozen/thawed more than 25 times.	
Use	This probemix allows the detection of aberrant methylation of one or more sequences of the <i>MGMT</i> gene as well as the presence of the <i>IDH1</i> (p.R132H=c.395G>A and p.R132C=c.394C>T) or <i>IDH2</i> (p.R172K=c.515G>A and p.R172M=c.515G>T) point mutations in a DNA sample. This probemix is designed for use only in combination with SALSA MLPA reagent kits, SALSA HhaI, and Coffalyser.Net as described in the MS-MLPA General Protocol.	
Quality control specifications	<ul style="list-style-type: none"> - Sufficient distance between peaks, absence of extra or shoulder peaks, and completeness of hybridisation and HhaI digestion of each individual probe, as tested on Applied Biosystems and Beckman/SCIEX GeXP sequencers. - Standard deviation of each individual probe ≤ 0.10, when tested on 23 different DNA samples of healthy individuals, extracted by various methods. - Each individual probe meets reaction-specific criteria when tested on a single DNA sample under various experimental conditions. - No DNA controls result in only five major peaks shorter than 121 nucleotides (nt): four Q fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height <25% of the median of the four Q fragments are not expected to affect MLPA reactions when sufficient (50-250 ng) sample DNA is used. Note: We observed one peak above the 25% threshold with length of approximately 132 nt in a No DNA control. 	<p>Test result</p> <p style="text-align: center; font-weight: bold;">PASS</p>

None of the ingredients are derived from humans, animals, or pathogenic bacteria. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for these products:** none of the preparations contain dangerous substances (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and amendments) at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments). If spills occur, clean with water and follow appropriate site procedures.

More information: www.mlpa.com ; www.mlpa.eu	
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SALSA MS-MLPA Probemix ME012 MGMT-IDH1-IDH2 sample picture

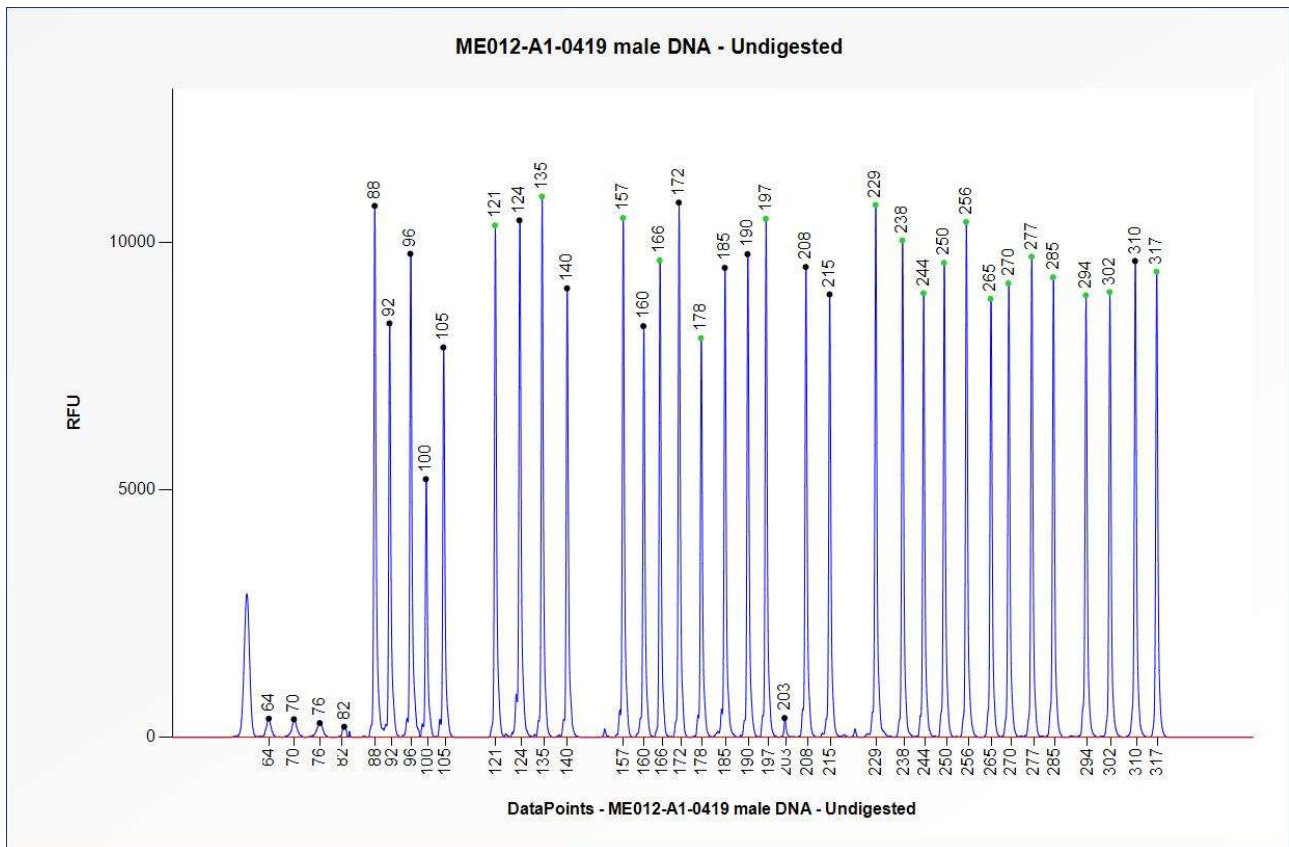


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng undigested human male control DNA analysed with SALSA MS-MLPA Probemix ME012 MGMT-IDH1-IDH2 (A1-0419) for the quantification of copy numbers.

Note: The *IDH1* mutation-specific probe 19529-L16492 at 203 nt might show very low unspecific background signal in wild-type samples. This background signal at 203 nt interferes with Coffalyser data analysis if the assignment of probe identities is not done properly. In order to identify each peak in the peak pattern as a probe, it is recommended to create a manual binset using the SD sample (SD054), which is provided with each vial of ME012 probemix. Instructions to create a manual binset can be found at Coffalyser.Net section in MRC-Holland Support Portal: <https://support.mlpa.com/>.

Certificate of Analysis

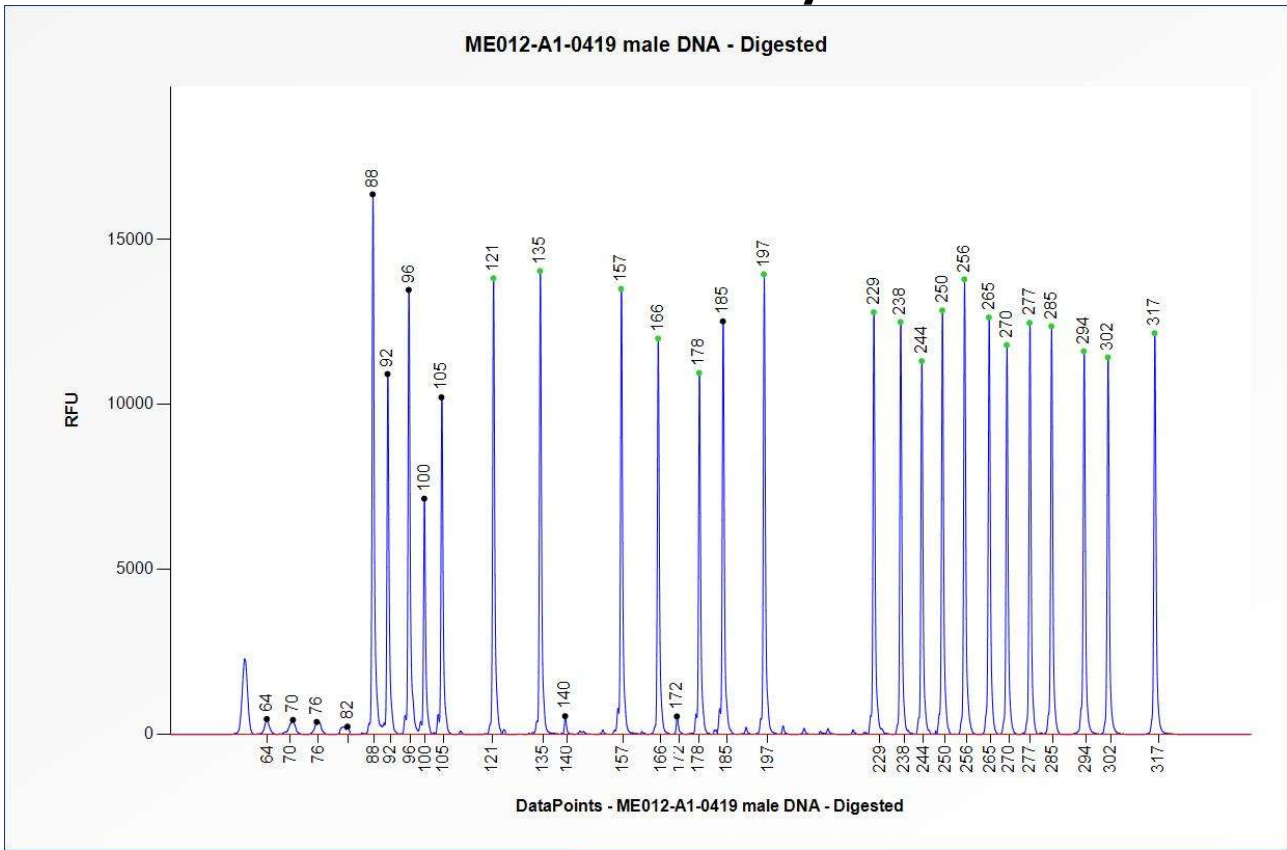


Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng digested human male control DNA analysed with SALSA MS-MLPA Probemix ME012 MGMT-IDH1-IDH2 (A1-0419) to determine the methylation status.

Certificate of Analysis

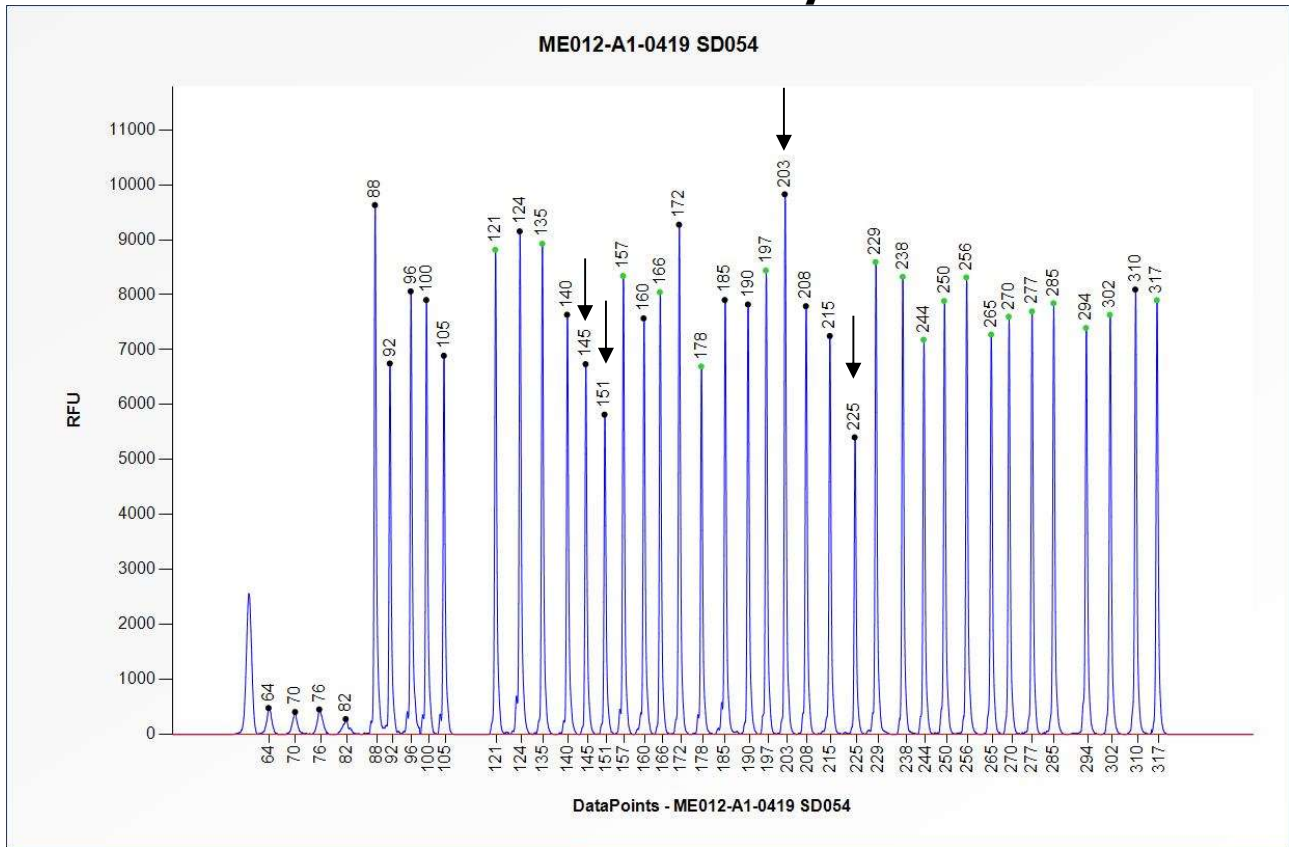


Figure 3. Capillary electrophoresis pattern from SALSA Binning DNA SD054-S01 (approximately 50 ng) analysed with SALSA MS-MLPA Probemix ME012 MGMT-IDH1-IDH2 (A1-0419). The locations of the *IDH1* p.R132H & p.R132C, and *IDH2* p.R172K & p.R172M probes at 203 & 225, and 145 & 151 nt are indicated.

This lot was certified by MRC-Holland on 31 July 2019.

This certificate is a declaration of analysis at the time of the manufacturing process. All assays were run in compliance with manufacturer's instructions for use.

Implemented changes in the COA

Version 02 – 10 June 2020 (02)

- Position of arrows adjusted for 145 and 151 nt mutation-specific probes in Figure 3.

Version 01 – 31 July 2019 (02)

- Not applicable, new document.