

## SALSA MS-MLPA KIT ME002-A1 Tumor suppressor-2

Lot 1207, 0407; 0107; 0706

Aberrant methylation of CpG-islands has been shown to be associated with transcriptional inactivation of tumor suppressor genes in a wide spectrum of human cancers. CpG-islands are located in or near the promoter region or other regulatory regions of approximately 50% of human genes.

This ME002-A1 MS-MLPA probemix contains 27 MS-MLPA probes detecting the methylation status of promoter regions of 25 different tumor suppressor genes. These tumor suppressor genes are frequently silenced by methylation in tumors, but are unmethylated in blood-derived DNA of healthy individuals. In addition, 14 reference probes are included which are not affected by *HhaI* digestion. Besides the detection of aberrant methylation, all 41 probes also yield information about copy number changes in the analyzed sample. The MLPA reaction requires as little as 20 ng of human DNA and can be used on a variety of DNA samples, including those derived from paraffin-embedded tissues. Please note that each MS-MLPA reaction generates two samples which need analysis capillary electrophoresis: one undigested sample for copy number detection and one digested sample for methylation detection. More information about MS-MLPA can be found on page 2 and in the MS-MLPA protocol.

The MS-MLPA probes in this ME002-A1 probemix detect sequences in promoter regions of tumor suppressor genes that are unmethylated in most blood-derived DNA samples. Upon digestion, the peak signal obtained in unmethylated samples will be very small or absent. In contrast, when tested on *in vitro* methylated human DNA, these probes do generate a signal. We have no data showing that methylation detected by a particular probe indeed influences the corresponding mRNA levels. Moreover, most MS-MLPA probes target only a single specific *HhaI* site of the CpG island. If methylation is absent for this particular CpG-site, it does not necessarily mean that the complete CpG island is unmethylated. For samples containing both tumor and normal cells, MLPA will indicate the average copy number of genes and the average methylation level.

This SALSA MS-MLPA kit can be used to detect *aberrant methylation* of one or more sequences of tumor suppressor genes. Methylation levels can be different for different tissues. Please use DNA derived from the same type of tissue and purified by the same method as reference sample. This SALSA MS-MLPA kit can be used to detect *deletions and duplications* of one or more sequences of the XYZ and ZYX genes. Heterozygous deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation by other methods. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Please note that most defects in these genes are expected to be small (point) mutations, most of which will not be detected by this MLPA test.

**SALSA® MS-MLPA® kits are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. This kit is not CE/FDA certified for use in diagnostic procedures. SALSA MLPA kits are supplied with all necessary buffers and enzymes. Purchase of the SALSA MLPA test kits includes a limited license to use these products for research purposes.**

The use of this MLPA kit requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002). The MS-MLPA method for the detection of both copy numbers, as well as, methylation changes was described in Nucleic Acid Research 33, e128 by Nygren et al. 2005.

### Related SALSA MLPA kits

– ME001 Tumor suppressor-1: Can be used for primary screening of ME002 tumor suppressor genes.

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### More information

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**References of SALSA MLPA kit ME001/ME002 Tumor suppressor**

- Henken FE. Et al. (2007). Sequential gene promoter methylation during HPV-induced cervical carcinogenesis. *Br J Cancer*. 2007 Oct 30.
- Worsham MJ. Et al. (2006). Epigenetic events of disease progression in head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg*. 2006 Jun;132(6):668-77.

**Methylation-specific MLPA**

A modification of the MLPA technique, MS-MLPA allows the detection of both copy number changes and unusual methylation levels of 10-50 different sequences in one simple reaction. MLPA probes for methylation quantification are similar to normal MLPA probes, except that the sequence detected by the MS-MLPA probe contains the sequence recognized by the methylation-sensitive restriction enzyme *HhaI*.

Similar to ordinary MLPA reactions, the MS-MLPA protocol starts with sample DNA denaturation and overnight hybridization. The reaction then is split into two tubes. One tube is processed as a standard MLPA reaction. This reaction provides information on copy number changes. The other tube of the MLPA hybridization reaction is incubated with the methylation-sensitive *HhaI* endonuclease while simultaneously, the hybridized probes are ligated. Hybrids of (unmethylated) probe oligonucleotides and unmethylated sample DNA are digested by the *HhaI* enzyme. Digested probes will not be exponentially amplified by PCR and hence will not generate a signal when analyzed by capillary electrophoresis. In contrast, if the sample DNA is methylated, the hemi-methylated probe-sample DNA hybrids are prevented from being digested by *HhaI* and the ligated probes *will* generate a signal. More information about MS-MLPA can be found in the MS-MLPA protocol.

**Data analysis**

The ME002-A1 Tumor suppressor-2 probemix contains 41 MLPA probes with amplification products between 136 and 481 nt. In addition, it contains 7 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, and three DNA denaturation control fragments (D-fragments) at 88-92-96 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Copy numbers (undigested sample)

Data of the undigested sample generated by this probemix should be normalized with a more robust method. The signals of all probes are normalized against every single probe separately, thereby creating as many ratios as there are probes. The median of all produced ratios gives an estimate of the final probe ratio, or ploidy status, of the sample's probe sequences in an MLPA mix. This way, the signal of each probe will be used as a normalization constant (population normalization). With the normalization constant, the ratio between reference and patient sample is determined for each probe.

Methylation analysis (digested and undigested sample)

Methylation status of a CpG-site for which an MS-MLPA probe is present is calculated by comparing A. the intra-normalized peak area of each MS-MLPA probe obtained on the digested patient sample with B. the intra-normalized peak area obtained on the digested reference DNA. Data generated by this probemix can be normalized by dividing the peak area of each MS-MLPA probe by the combined areas of the nearest reference probes. Secondly, the relative peak area of each target probe from the digested sample should be divided by that obtained in the undigested sample resulting in a probe ratio. Multiplying this value with 100 gives a estimation of the percentage of methylation.

Aberrant methylation can be identified by the appearance of a signal peak after *HhaI* digestion that was absent in the digested reference DNA. Using all reference probes for normalization generally gives good results, but when there is a clear difference in size to signal drop between digested and undigested samples, division using the nearest reference probes only is recommended.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website [www.mlpa.com](http://www.mlpa.com).

**Note:** A MS-MLPA probe is directed to one specific *HhaI* site in a CpG island, if methylation is absent for a particular CpG-site, this does not necessarily mean that the whole CpG island is unmethylated! Samples

containing both tumor as well as normal cells, MLPA experiments will indicate the average copy number of genes.

**Some probes, such as CDH13 (220 nt), ESR1 (301 nt) and MGMT (382 nt), show background signals in DNA derived from blood, but NOT in from isolated DNA from other tissues! These background signals vary between 10-20%! (see figure 2 and 3 below).**

## SALSA MS-MLPA ME002-A1 tumor suppressor probemix

Length (nt)	SALSA MLPA probe	Hha1 site	Gene detected	Chromosomal position
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA			
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation			
136	0981-L0566		CREM	10p12.1
142	3296-L1269	+	BRCA1	17q21
148	2285-L1776	+	BRCA2	13q12.3
154	3366-L2750		PARK2	06q26
160	3023-L2413	+	ATM	11q23
166	2374-L2530	+	TP53	17p13.1
175	3708-L3162		PTCH	09q22.3
184	3808-L2169	+	PTEN	10q23.3
193	5670-L5146	+	MGMT	10q26
202	1245-L0793		MLH3	14q24.3
211	3750-L3210	+	PAX5	09p13
220	2257-L1742	+	CDH13	16q24.2
229	2334-L1820		PAH	12q23
238	1684-L1264	+	TP73	01p36
247	2755-L2204	+	WT1	11p13
256	1462-L0927		PMP22	17p12
265	3818-L3850	+	VHL	03p25.3
274	2747-L2174	+	GSTP1	11q13
283	1832-L1397		TSC2	16p13.3
292	2737-L2164	+	CHFR	12q24.33
301	2746-L2173	+	ESR1	06q25.1
310	3033-L2588		TNXB	06p21.3
319	2734-L2161	+	RB1	13q14.2
328	1250-L0798	+	MSH6	02p16
337	1700-L1341		APC	05q22
346	1678-L1258	+	THBS1	15q15
355	3816-L1179	+	IGSF4	11q23
364	3638-L2945		PTEN	10q23.3
373	6783-L2167	+	STK11	19p13.3
382	1681-L1261	+	MGMT	10q26
391	2184-L1682		PARK2	06q26
400	2252-L1737	+	PYCARD	16p12
409	3749-L3209	+	PAX6	11p13
418	2670-L2137		ATM	11q23
427	1530-L3851	+	CDKN2A	09p21
436	3752-L3212	+	GATA5	20q13.33
445	0627-L0183		IL2	04q26
454	4046-L2172	+	RARB	03p24
463	4500-L2761	+	CD44	11p12
472	4502-L2199	+	RB1	13q14.2
481	2683-L2148		CASR	03q21

**Note:** PAX5 was named PAX5a in the previous product description. PYCARD was named ASC in the previous product description. Please notify us of any mistakes: [info@mlpa.com](mailto:info@mlpa.com).

This probemix was developed by A. Errami & J.P. Schouten at MRC-Holland. In case the results obtained with this probemix lead to a scientific publication, it would be very much appreciated if the probemix designer could be made a coauthor. Info/remarks/suggestions for improvement: [info@mlpa.com](mailto:info@mlpa.com).

## ME002-A1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Hha1 site	MV 35	Chromosomal position
238*	1684-L1264	TP73	+	01-003.6	01p36
328**	1250-L0798	MSH6		02-047.9	02p16
454*	4046-L2172	RARB	+	03-025.5	03p24
265*	3818-L3850	VHL	+	03-010.2	3p26-p25
481	2683-L2148	CASR		03-123.4	03q21
445	0627-L0183	IL2		04-123.7	04q26-q27
337	1700-L1341	APC		05-112.2	05q22
310	3033-L2588	TNXB		06-032.1	06p21.3
301	2746-L2173	ESR1	+	06-152.4	06q25.1
391	2184-L1682	PARK2		06-162.4	06q26
154	3366-L2750	PARK2		06-162.4	06q26
211	3750-L3210	PAX5	+	09-036.9	09p13
427*	1530-L3851	CDKN2A	+	09-021.97	09p21
175	3708-L3162	PTCH	+	09-095.3	09q22.3
136	0981-L0566	CREM		10-035.50	10p12.1
184*	3808-L2169	PTEN	+	10-089.7	10q23.3
364	3638-L2945	PTEN		10-089.7	10q23.3
382**	1681-L1261	MGMT	+	10-131.3	10q26
193**	5670-L5146	MGMT	+	10-131.3	10q26
409	3749-L3209	PAX6	+	11-031.8	11p13
463*	4500-L2761	CD44	+	11-035.2	11p12
247	2755-L2204	WT1	+	11-032.4	11p13
274*	2747-L2174	GSTP1	+	11-067.1	11q13
418*	2670-L2137	ATM		11-107.7	11q23
355*	3816-L1179	IGSF4	+	11-114.7	11q23
160	3023-L2413	ATM	+	11-107.7	11q23
229	2334-L1820	PAH		12-101.8	12q23
292	2737-L2164	CHFR	+	12-132.05	12q24.33
148*	2285-L1776	BRCA2	+	13-031.8	13q12.3
472	4502-L2199	RB1	+	13-047.9	13q14.2
319	2734-L2161	RB1	+	13-047.9	13q14.2
202	1245-L0793	MLH3		14-074.6	14q24.3
346	1678-L1258	THBS1	+	15-037.7	15q15
400	2252-L1737	PYCARD	+	16-031.1	16p12
283	1832-L1397	TSC2		16-002.1	16p13.3
220*	2257-L1742	CDH13	+	16-081.2	16q24.2
256	1462-L0927	PMP22		17-015.1	17p12
166	2374-L2530	TP53	+	17-007.5	17p13.1
142*	3296-L1269	BRCA1	+	17-038.5	17q21
373	6783-L2167	STK11	+	19-001.16	19p13.3
436	3752-L3212	GATA5	+	20-060.5	20q13.33

\* These genes have a second probe in SALSA MLPA kit ME001 Tumor suppressor-1, recognizing a different CpG-site.

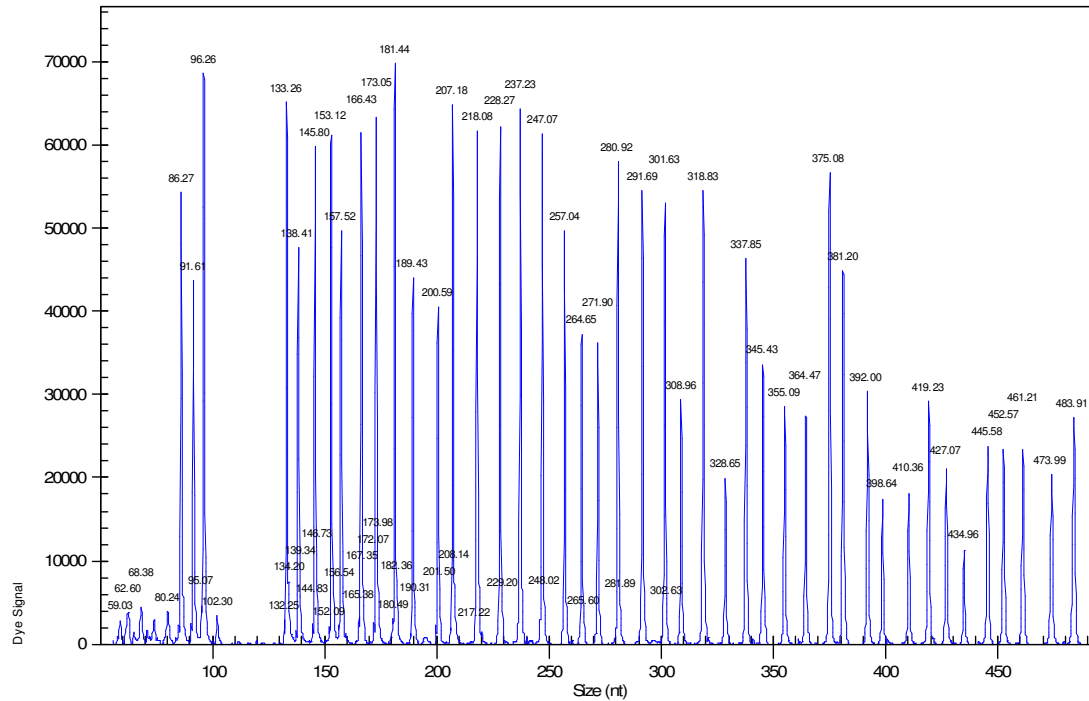
\*\* More methylation probes for MGMT and MSH6 are available in SALSA MLPA kit ME011 MMR.

The Mapview35 location is the distance in Mb towards the P-telomere according to the NCBI database MapView Build 35, January 2005. 22-031.6 = 31.6 Mb from the P-telomere of chromosome 22.

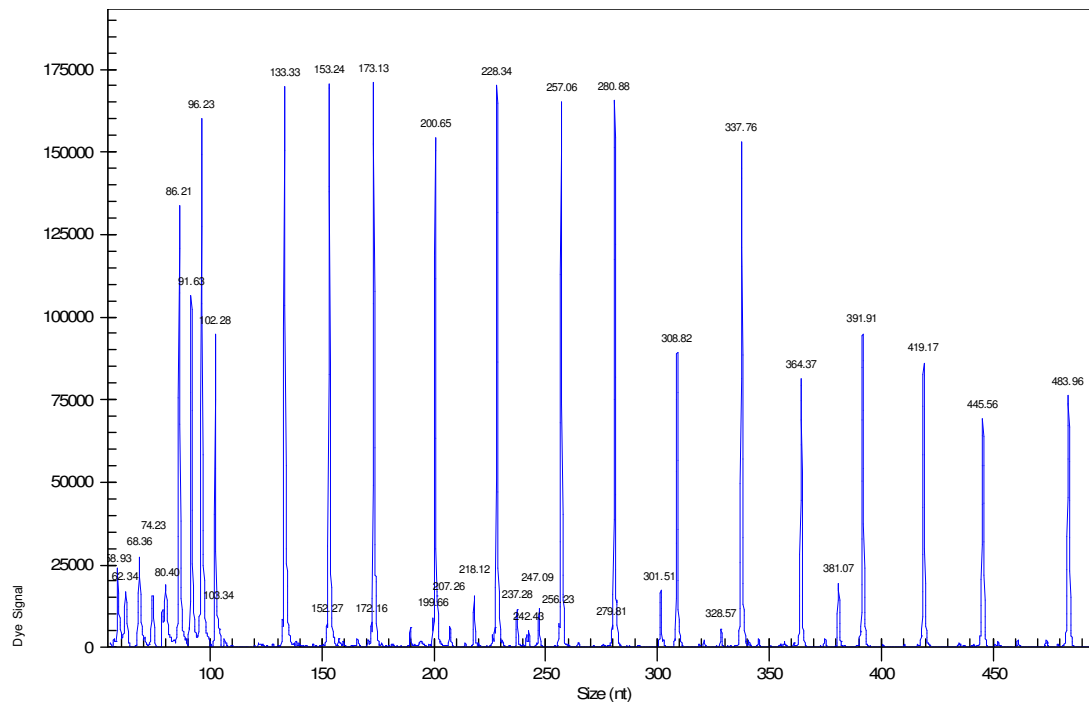
Please notify us of any mistakes: [info@mlpa.com](mailto:info@mlpa.com).



## SALSA MLPA kit ME002-A1 Tumor suppressor-2 sample pictures



**Figure 1.** Capillary electrophoresis pattern from a sample of approximately 50 ng undigested human male control DNA analyzed with SALSA MLPA kit ME002-A1 Tumor suppressor-2 (lot 0407).



**Figure 2.** Capillary electrophoresis pattern from a sample of approximately 50 ng digested human male control DNA analyzed with SALSA MLPA kit ME002 Tumor suppressor-2 (lot 0407).

Using control Promega DNA all the methylation-sensitive probes are digested and thus no amplification products are detected of the methylation probes. However, three probes show some background signals, as they are not completely digested when DNA derived from BLOOD is used (probes CDH13-220bp, ESR-301 bp and MGMT-382 nt). However, when DNA from other types of tissues is used, these probes will be completely digested and no signal will be detected.