Certificate of Analysis
SALSA® MLPA® Probemix P047 RB1

Catalogue #	**P047-025R, P047-050R, P047-100R**
Product name	**Probemix P047 RB1**
LOT	**E2-0824**

Σ	25, 50, or 100 reactions.	
Shipping conditions	Dry ice or cooling elements.	
	Store upon arrival between -25°C and -15°C.	
	Expiration date: August 2029 when stored at recommended conditions. This product should not be frozen/thawed more than 25 times.	
Purpose	This product has been developed for the detection of deletions or duplications in the *RB1* gene and of methylation status of the *RB1* gene promoter and imprinted locus. This probemix is designed for use only in combination with SALSA MLPA reagent kits, SALSA HhaI and Coffalyser.Net analysis software as described in MLPA General Protocol and in MS-MLPA General Protocol.	
Quality control specifications	- 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 peak in the range of 0-40 nt 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.	Test result PASS

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.

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Certificate of Analysis
SALSA MLPA Probemix P047-E2 RB1 sample pictures

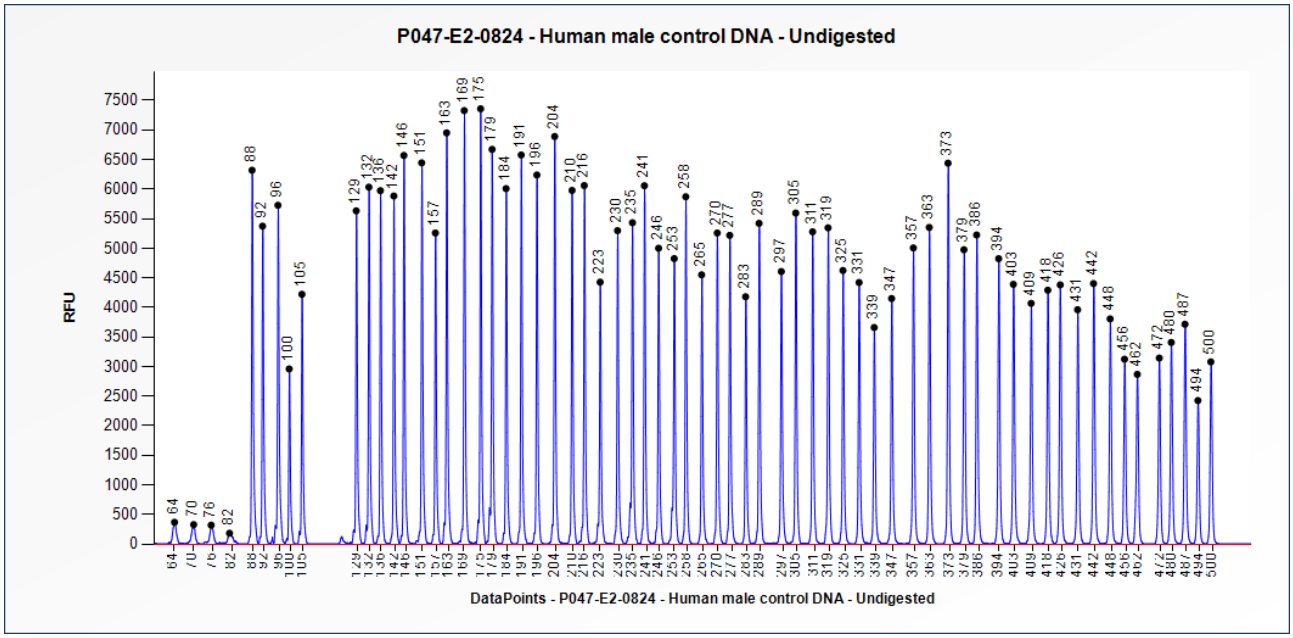

Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA Probemix P047 RB1 (E2-0824) for the quantification of copy numbers.

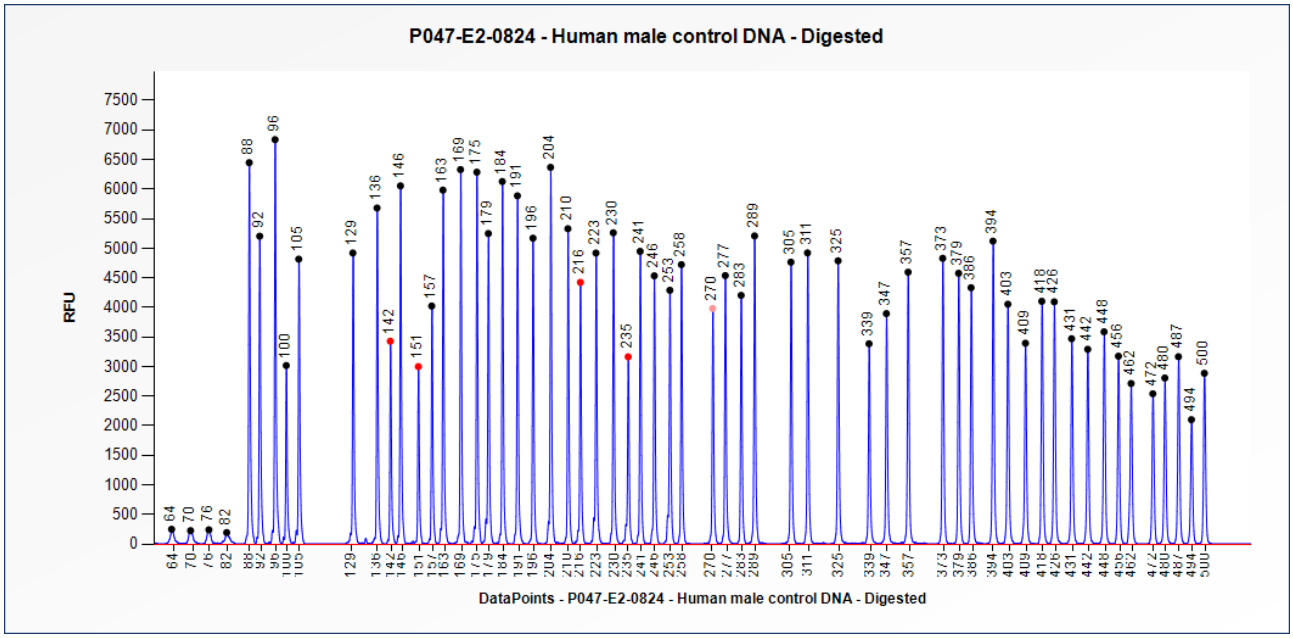

Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng digested human male control DNA analysed with SALSA MLPA Probemix P047 RB1 (E2-0824) to determine the methylation status of CpG106 promoter region (digested probe peaks at 265, 297, 319, 331 and 363 nt are absent) and CpG85 imprinted region (digested probe peaks at 142, 151 and 235 nt have reduced height). The signal for 132 nt digestion control probe is absent.

This lot was certified by MRC Holland on 04 March 2025.

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 01 – 04 March 2025 (4) - Not applicable, new document.