Certificate of Analysis
SALSA® MLPA® Probemix P520 MPN mix 2

Catalogue #	**P520-025R, P520-050R, P520-100R**
Product name	**Probemix P520 MPN mix 2**
LOT	**A2-1123**

Σ	25, 50, or 100 reactions.	
Shipping conditions	Dry ice or cooling elements.	
	Store upon arrival between -25°C and -15°C.	
	Expiration date: November 2028 when stored at recommended conditions. This product should not be frozen/thawed more than 25 times.	
Purpose	This product has been developed to determine the presence (with ≥1 % allele burden) of the following mutations: *JAK2* p.V617F, *JAK2* p.N542-E543del, *JAK2* p.E543-D544del, *MPL* p.W515K, *MPL* p.W515L, *CALR* p.L367fs*46, *CALR* p.K385fs*47 and *KIT* p.D816V, as described in Table 1 and 2 of the product description. This probemix is designed for use only in combination with SALSA MLPA reagent kits, SD057 and Coffalyser.Net analysis software as described in the MLPA General Protocol.	
Quality control specifications	- Sufficient distance between peaks, absence of extra or shoulder peaks, and completeness of hybridisation of each individual probe, as tested on Applied Biosystems and Beckman/SCIEX GeXP sequencers. - Standard deviation of each individual probe ≤0.20, when tested on 23 different DNA samples of healthy individuals, extracted by various methods. - 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 (100-400 ng) sample DNA is used. - Note: We observed in some normal female DNA controls a low peak with length of approximately 105 nt - same length as Y chromosome specific probe. This unspecific peak is in some cases size-called and could hinder the correct determination of the gender in Coffalyser.Net.	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 P520-A2 MPN mix 2
sample pictures

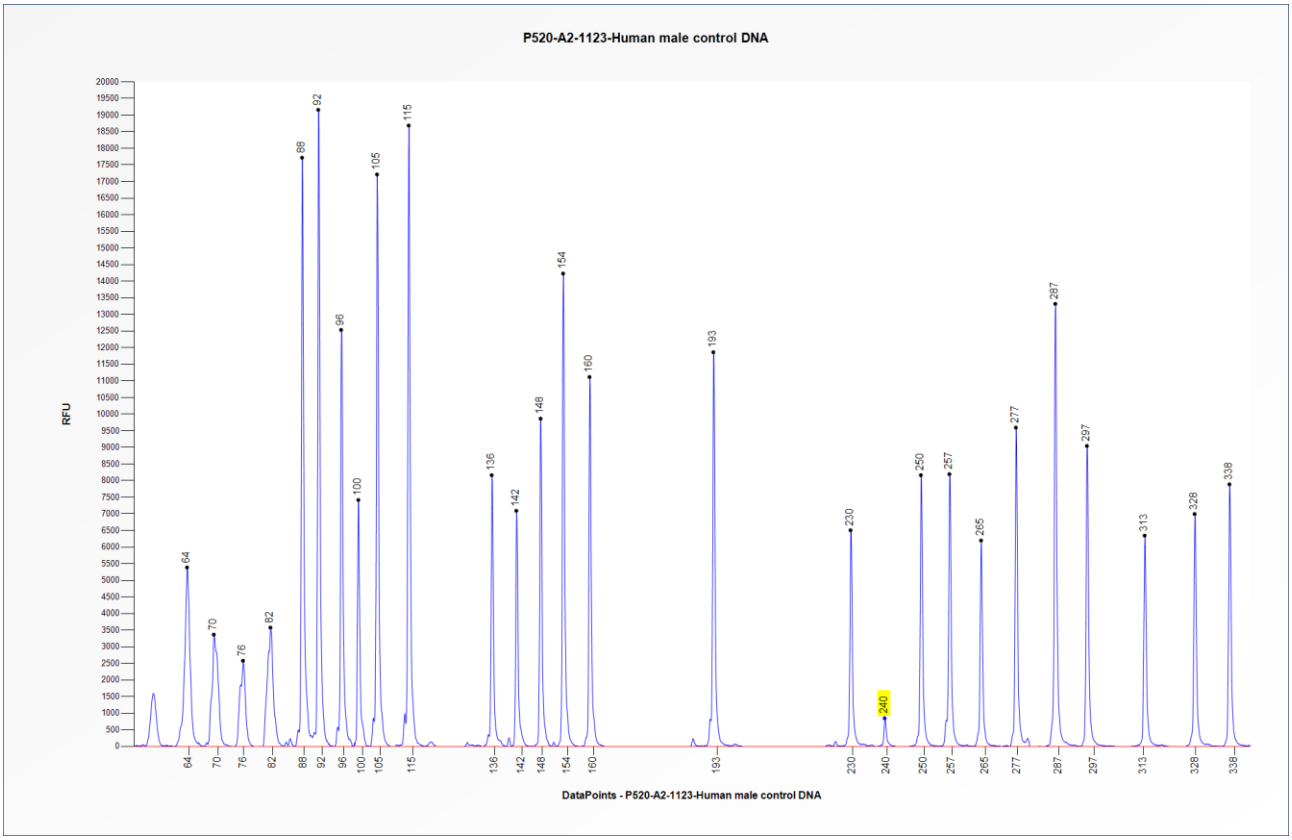

Figure 1. Capillary electrophoresis pattern from a sample of approximately 100 ng human male control DNA analysed with SALSA MLPA Probemix P520 MPN mix 2 (A2-1123).

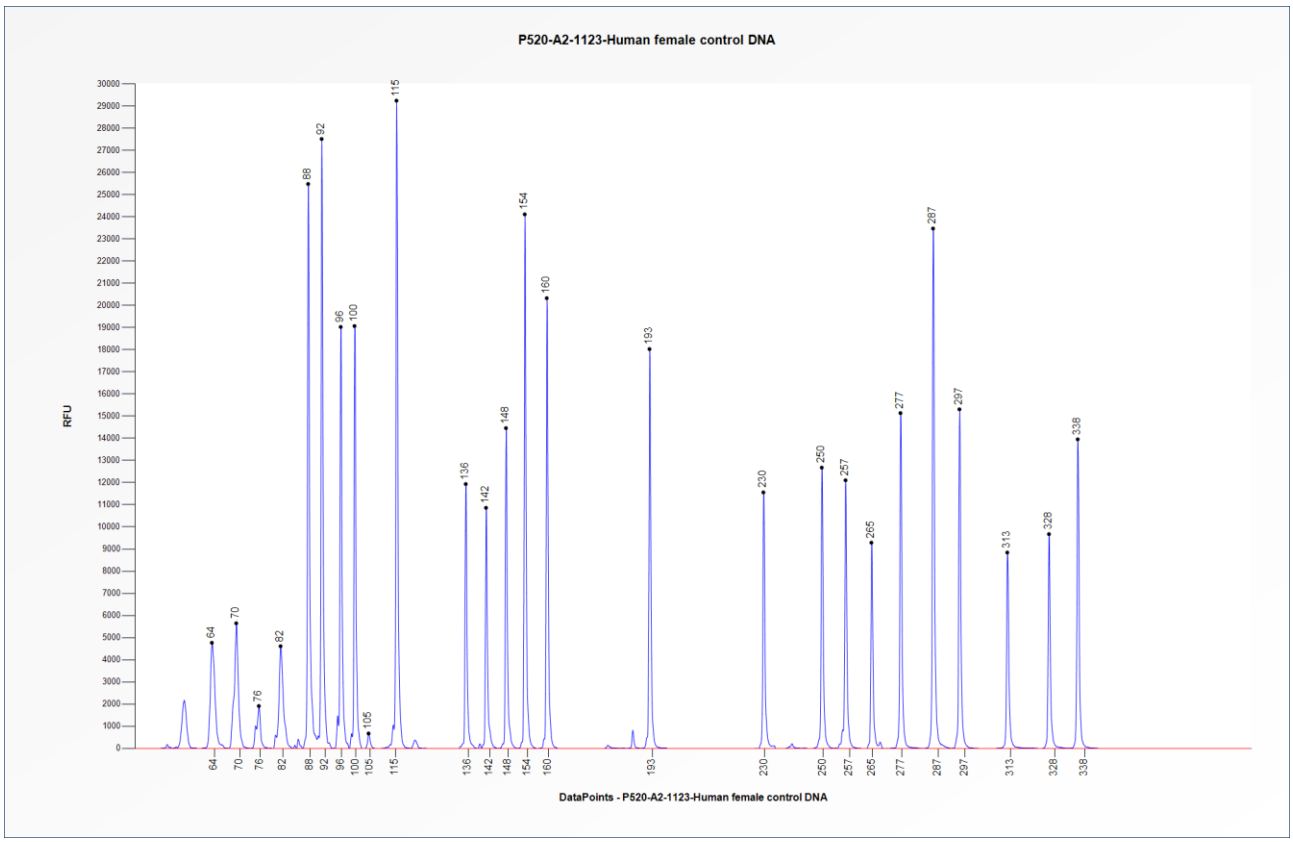

Figure 2. Capillary electrophoresis pattern from a sample of approximately 100 ng human female control DNA analysed with SALSA MLPA Probemix P520 MPN mix 2 (A2-1123).

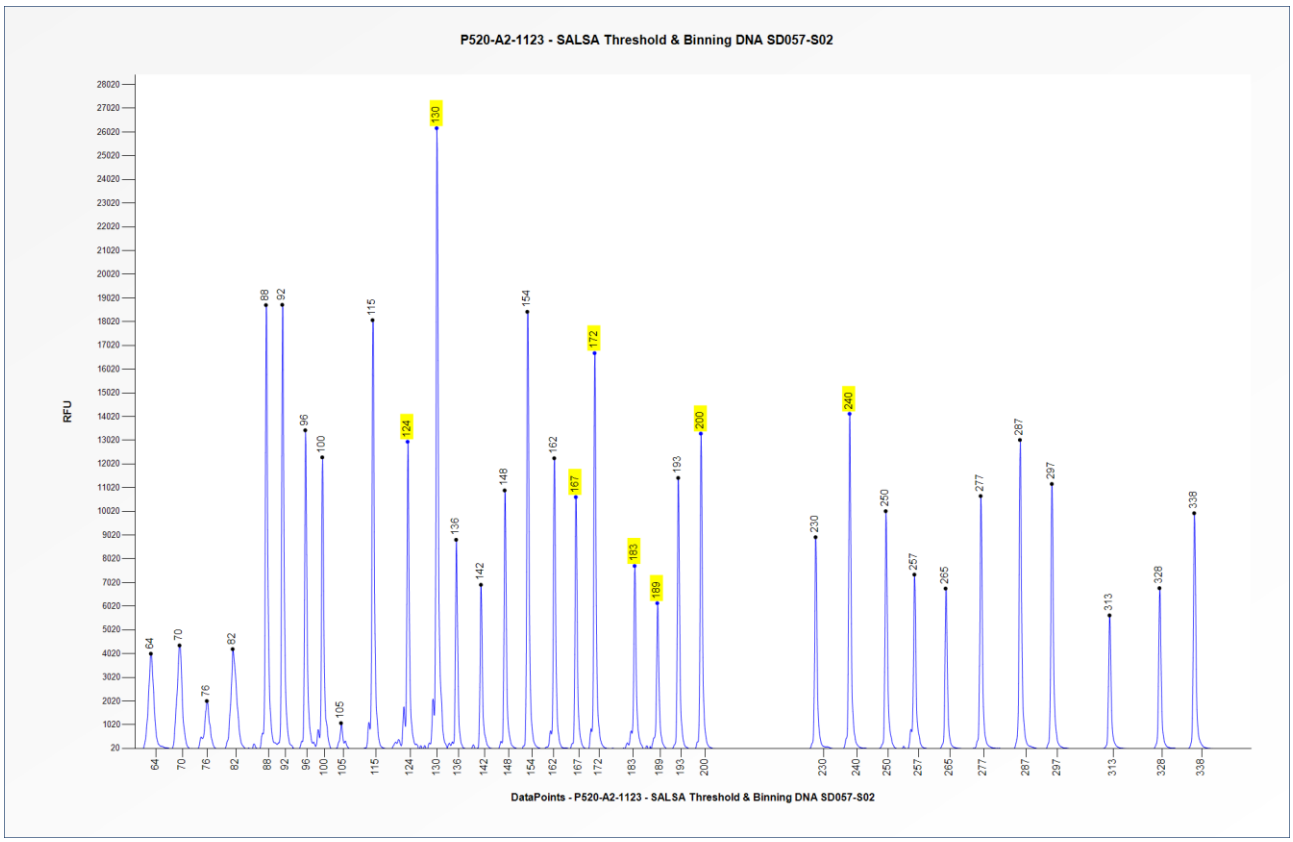

Figure 3. Capillary electrophoresis pattern from SALSA Threshold & Binning DNA SD057-S02 (approximately 100 ng) analysed with SALSA MLPA Probemix P520 MPN mix 2 (A2-1123). The location of the mutation-specific probes at 124 nt (*CALR* p.L367fs*46), 130 nt (*CALR* p.K385fs*47), 167 nt (*JAK2* p.N542-E543del), 172 nt (*JAK2* p.E543-D544del), 181 nt (*MPL* p.W515K), 186 nt (*MPL* p.W515L), 200 nt (*KIT* p.D816V) and 240 nt (*JAK2* p.V617F) is indicated.

This lot was certified by MRC Holland on 03 July 2024.

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– 21 May 2025 (6) - The SD name has been adjusted to SALSA Threshold & Binning DNA SD057.