

Product Description SALSA® Binning DNA SD057-S01

Version S01.

Catalogue number: SD057: SALSA® Binning DNA, 20 reactions

NOTE: Binning DNA SD057-S01 contains an estimated allele burden of 0.5-1% for each mutation listed in Table 1. The concentration of SD057-S01 is 20 ng/µl (instead of 10 ng/µl in other Sample DNAs used for MLPA reactions). A total of 100 ng SD057 per MLPA reaction with P520-A2 is used.

NOTE: This Binning DNA should NOT be used for quantification of the mutation burden, only as a qualitative aid for determining presence or absence of the mutation.

To be used with the following SALSA MLPA probemix: P520-A2 MPN mix 2, in combination with a SALSA® MLPA® reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Precautions and warnings: For professional use only. Always consult the most recent product description AND the corresponding probemix product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

Intended use: This SD057 DNA can be used as Binning DNA sample for the MLPA probemix version as specified above and in Table 1. for the binning of eight mutation-specific probes detecting mutations in the CALR gene (p.L367fs*46=c.1092_1143del52 and p.K385fs*47=c.1154_1155insTTGTC), the JAK2 gene (p.N542_E543del=c.1624_1629delAATGAA, p.E543_D544del=c.1627_1632delGAAGAT and p.V617F=c.1849G>T), the MPL gene (p.W515K=c.1543_1544TG>AA and p.W515L=c.1544G>T) and KIT gene (p.D816V=c.2447A>T). Binning and filtering are the processes of linking a signal to its probe identity by use of the probe length. For all above mentioned mutation-specific probes, this Binning DNA can also be used as an artificial positive control for the corresponding mutations.

Please note that this Binning DNA is a mixture of female genomic DNA from healthy individuals and artificial DNAs of 50-80 nt length and not covering the whole exon.

This product is for research use only (RUO).

Experimental set up: MLPA reactions for binning purposes should be performed with 5 µl of Binning DNA, properly mixed. Inclusion of one reaction with SALSA Binning DNA SD057 in each MLPA experiment is essential as it can aid in data binning of the peak pattern using Coffalyser.Net software and in mutation calling. Both the MLPA reaction and analysis of results should be performed according to the instructions described in the General Protocol for Multiplex Ligation-dependent Probe Amplification (MLPA®), with the exception that for PCR reaction 40 PCR cycles (instead of 35 PCR cycles) should be used in step 9 for the P520-A2 probemix. **More detailed information about the protocol and the probemix can be found in the probemix product description.**

Data analysis: Coffalyser.Net software must be used for analysis of MLPA experiments. When performing the fragment analysis step in Coffalyser.Net, select the *bin smpl*-column for the SD057 sample. By selecting the SD057 sample as your binning sample, probes will be correctly identified in the peak pattern across all samples. Coffalyser.Net software is available free of charge on www.mlpa.com.

Binning DNA content: MRC-Holland is unable to provide mutation-positive human DNA samples. As an alternative, we have prepared a mixture of female genomic DNA from healthy individuals and a titrated amount of synthetic DNA that contains the target sequences recognised by the mutation-specific probes present in the MLPA probemix versions as specified above and in Table 1.

The synthetic DNA included in the SD057 DNA contains partial sequences of the CALR, JAK2, MPL and KIT genes. These sequences will be detected by eight MLPA probes that are present in the P520-A2 probemix

(for details, see Table 1) and will therefore generate a signal for these mutation-specific probes. The amount of synthetic DNA present mimics the signal obtained on samples that contain an estimated mutation allele burden of 0.5-1%.

Storage and stability: Upon arrival, Binning DNA must be stored between -25 °C and -15 °C, in the original packaging. When stored under the recommended conditions, a shelf life of at least 1 year is guaranteed, also after opening. The expiry date is mentioned on the label of the vial.

Table 1. Mutation-specific probe targets in SD057-S01 Binning DNA

Product	Gene/Exon	Probe length	Probe ID	Present in probemix version	Details
P520	MPL exon 10	185 nt	S1048-SP0405-L29871	A2	p.W515L=c.1544G>T
	MPL exon 10	179 nt	S1048-SP0405-L29870	A2	p.W515K=c.1543_1544TG>AA
	KIT exon 17	200 nt	17722-SP0542-L23707	A2	p.D816V=c.2447A>T
	JAK2 exon 12*	167 nt	16924-L21237	A2	p.N542_E543del=c.1624_1629delAATGAA
	JAK2 exon 12*	172 nt	16924-L21238	A2	p.E543_D544del=c.1627_1632delGAAGAT
	JAK2 exon 14**	240 nt	13190-L21572	A2	p.V617F=c.1849G>T
	CALR exon 9	124 nt	S0999-L26702	A2	p.L367fs*46=c.1092_1143del52
	CALR exon 9	130 nt	S1001-L26517	A2	p.K385fs*47=c.1154_1155insTTGTC

* When both probe signals at 167 nt and at 172 nt are present in the peak pattern it is indicative for JAK2 p.E543_D544del mutation. When only the probe signal at 167 nt is present, it is indicative for JAK2 p.N542_E543del mutation. SD057 contains only the JAK2 p.E543_D544del mutation sequence, but this also generates a signal at 167 nt.

** This probe might give a small background signal when tested on normal DNA samples not carrying the JAK2 p.V617F mutation. Please, carefully examine the raw data and compare it with the SD057-S01 sample to avoid false positive results when analysing this probe signal.

Note: Mutation nomenclature and exon numbering used here may differ from literature! Please notify us of any mistakes: info@mlpa.com.

More information: www.mlpa.com; www.mlpa.eu

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Implemented Changes – compared to the previous SD057 product description versions

- Version S01-04 – 18 October 2018 (15)*
- SD057 is only to be used for binning and mutation calling purposes, NOT to be used for quantification of the mutation burden.
 - Information regarding the estimated mutation burden of SD057 has been changed from 1% to 0.5-1%.
- Version S01-03 – 26 September 2017 (15)*
- Modified the length of MPL exon 10 probe in Table 1.
- Version S01-02 – 14 August 2017 (15)*
- SD lot number removed.
 - Updated version of P520 probemix.
 - Instructions for use adjusted for semi-quantitative determination of allelic burden.
 - Various textual and layout changes.
- Version 01 – 11 September 2015 (10)*
- Not applicable, new document.