Product Description SALSA® Binning DNA SD069-S02

Version S02. Compared to previous version S01, plasmid DNA is used instead of synthetic DNA.

Catalogue number: SD069: SALSA® Binning DNA**,** six reactions

To be used with the following SALSA MLPA probemix: P420-B1 MPN mix 1, 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 SD069 DNA can be used as Binning DNA sample for the MLPA probemix version as specified above and in Table 1. Binning and filtering are the processes of linking a signal to its probe identity by use of the probe length. The Binning DNA can also be used as an artificial positive control for the specific mutations, except for the JAK2 N542_E543del mutation-specific probe. See Table 1 and the corresponding probemix product description for more details on mutation-specific probe targets present.
Please note that this Binning DNA is a mixture of female genomic DNA from healthy individuals and artificial DNAs of 50-80 nt length covering probe target sequences 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 SD069 in the initial MLPA experiment is essential as it can aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when a different polymer type is used).

Data analysis: Coffalyser.Net software must be used for analysis of MLPA experiments. When performing the fragment analysis step in Coffalyser.Net, select SD069 in the *bin smpl* –column. By selecting the SD069 sample as your binning sample, probes will be correctly identified in the peak pattern across all patient samples. Coffalyser.Net software is available free of charge on www.mlpa.com.

Warning: Binning DNA should never be used as a reference sample in the MLPA data analysis. Neither should it be used in quantification of mutation signal(s), as for this purpose true mutation positive patient samples or cell lines should be used. It is strongly advised to use sample and reference DNA extracted with the same method and derived from the same source of tissue.

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 plasmid DNA that contains the target sequences recognised by the mutation-specific probes present in the MLPA probemix version as specified above and in Table 1.

The plasmid DNA included in the SD069 DNA contains partial sequences of the *CALR*, *JAK2*, *KIT* and *MPL* genes. These sequences include eight different mutations which will be detected by MLPA probes that are present in the aforementioned probemix version (for details, see Table 1) and will generate mutation-specific signals for these probes.
Please note that the plasmid DNA contains the target sequences detected by the above mentioned probes and the sequence of the 105 nt chromosome Y specific control fragment. The amount of plasmid DNA in this Binning DNA (relative to the genomic DNA) results in a relative probe signal for the 105 nt probe on this female DNA which is similar to the relative probe signal obtained on male DNA samples. As a result, the 100 and 105 nt control fragments indicate the presence of two copies chromosome X and one copy chromosome Y.

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 SD069-S02 Binning DNA

Probemix	Gene/Exon	Probe length	Probe ID	Present in probemix version	Details
P420	MPL exon 10	186 nt	S1048-SP0405-L29871	B1	p.W515L= c.1544G>T
	MPL exon 10	181 nt	S1048-SP0405-L29870	B1	p.W515K= c.1543_1544TG>AA
	KIT exon 17	200 nt	17722-SP0542-L23707	B1	p.D816V= c.2447A>T
	JAK2 exon 12 ~	167 nt	16924-L21237	B1	p.N542_E543del= c.1624_1629delAATGAA
	JAK2 exon 12 ~	172 nt	16924-L21238	B1	p.E543_D544del= c.1627_1632delGAAGAT
	JAK2 exon 14	240 nt	13190-L21572	B1	p.V617F= c.1849G>T
	CALR exon 9	124 nt	S0999-L26702	B1	p.L367fs*46= c.1092_1143del52
	CALR exon 9	130 nt	S1001-L26517	B1	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.

Note: Mutation nomenclature and exon numbering used here may differ from literature! Please notify us of any mistakes: info@mlpa.com. Please consult the respective probemix product description to find corresponding gene transcripts.

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200

Implemented Changes – compared to the previous SD069 product description versions

Version S02-01 – 17 February 2021 (15)
- Product description adapted to a new version of SD069.
- Details about the SD069 adjusted: plasmid DNA used instead of synthetic DNA.
- Minor textual and layout changes in Table 1.
- Small changes of probe lengths in Table 1 in order to better reflect the true lengths of the amplification products.

Version S01-02 – 19 September 2017 (15)
- Product description adapted to new probemix version in text on page 1 and in table 1.
- Information about JAK2 N542_E543del mutation-specific probe added at 'intended use' on page 1.
- Removed information about reliable mutation detection for MPL W515L mutation from table 1.
- Minor textual and layout changes.

Version S01-01 – 02 June 2017 (15)
- Not applicable, new document.