Product Description SALSA® Binning DNA SD030-S01

Version S01.

Catalogue number: SD030: SALSA® Binning DNA, six reactions

To be used with the following SALSA MLPA probemixes: P116-B1/B2 SGC; P199-B2/B3 HEXA; P255-B1 ALDOB-FBP1; P285-C3 LRP5 and P305-B2 AGXT, 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 or the MS-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 SD030 DNA can be used as Binning DNA sample for the MLPA probemix versions 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 point mutations, except for the *ALDOB* 448G>C 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 DNA 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 SD030 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 SD030 in the *bin smpl* –column. By selecting the SD030 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 versions as specified above and in Table 1.

The plasmid DNA included in the SD030 DNA contains partial sequences of the *AGXT*, *ALDOB*, *LRP5*, *FKRP* and *HEXA* genes. These sequences include nine different mutations which will be detected by MLPA probes that are present in the aforementioned probemix versions (for details, see Table 1) and will generate mutation-specific signals for these probes.
Please note that the plasmid 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 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 SD030-S01 Binning DNA

Probemix	Gene/Exon	Probe length	Probe ID	Present in probemix version	Details
P116	*FKRP* / exon 4	259 nt	11373-L13479	B1, B2	c.826C>A; p.L276I
P199	*HEXA* / exon 11	166 nt	06722-L06309	B2, B3	c.1278insTATC
	HEXA / exon 12	172 nt	06724-L06312	B2, B3	IVS12+1G>C
P255	*ALDOB* / exon 5*	328 nt	08669-L08680	B1	c.448G>C; p.A149P
	ALDOB / exon 5	355 nt	08670-L08682	B1	c.524C>A; p.A174D
P285	*LRP5* / exon 3	202 nt	09270-SP0044-L09500	C3	c.512G>T; p.G171V
P305	*AGXT* / exon 1	264 nt	09734-L10145	B2	c.33_34insC
	AGXT / exon 4	283 nt	09740-L10150	B2	c.508G>A; p.G170R
	AGXT / exon 7	229 nt	09743-L10154	B2	c.731T>C; p.I244T

*Contrary to the other mutation-specific probes, Binning DNA SD030-S01 is expected to generate a higher signal as compared to a true mutation positive sample for this probe. As a result, this Binning DNA can only be used for binning of this mutation-specific probe, not as an artificial positive control.

Note: Mutation nomenclature and exon numbering used here may differ from literature! Please notify us of any mistakes: info@mlpa.com. Corresponding gene transcripts can be found in the respective probemix product descriptions.

More information: www.mlpa.com; www.mlpa.eu	
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Implemented Changes – compared to the previous SD030 product description versions

Version S01-07 – 12 February 2019 (15)
- Information about P199-B3 added on page 1 and in Table 1.

Version S01-06 – 09 January 2019 (15)
- Product description was adapted to new P116 FKRP and P285 LRP5 versions.
- Information about P103 mix removed from page 1 and from Table 1.
- Amino acid change included in detail column of Table 1.
- Minor format changes on page 1.

Version S01-05 – 17 May 2018 (15)
- Information about P285-C3 added on page 1 and in Table 1.
- Information about P103-B1, P199-B1 and P285-C1 removed on page 1 and in Table 1.

Version S01-04 – 08 March 2017 (15)
- Information about P305-B1 and P193-A1/A2 probemixes removed on page 1 and in Table 1.
- Precautions and warnings added on page 1.
- Information corrected of mutation-specific probe in P199, detecting 1278insTATC mutation.
- Various minor textual and layout changes.

Version 03 (14) – 30 August 2016
- Version number of probemixes added to *intended use* section on page 1.
- Exon numbering adjusted of mutation-specific probe in P103, detecting IVS14=1G>A mutation.
- Information about P199-B2 probemix added in text on page 1 and Table 1.

- Table 1 updated and explanation on when to use Binning DNA in the MLPA experiment added on page 1.
- Information on where to find corresponding gene transcripts added to note at Table 1.
- Lot number removed throughout document.
- Various minor textual and layout changes.

Version 02 (08)

- Minor textual changes on page 1 and 2.
- Information about P255-B probemix added in text on page 1 and Table 1.
- Minor textual and format changes in Table 1 and Note added below.

Version 01 (02)

- Not applicable, new document.