Product Description SALSA® Binning DNA SD006-S01

Version S01.

Catalogue number: SD006: SALSA® Binning DNA**,** six reactions

To be used with the following SALSA MLPA probemix: P315-B1 EGFR 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 SD006 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 point mutations. 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 SD006 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 SD006 in the *bin smpl* –column. By selecting the SD006 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/SNP 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 SD006 DNA contains partial sequences of the EGFR gene. These sequences include two 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 a 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 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 SD006-S01 Binning DNA

Probemix	Gene /Exon	Probe length	Probe ID	Present in probemix version	Details
P315	EGFR exon 20 (22)	246 nt	17162-SP0448-L21565	B1	c.2369C>T=p.T790M
	EGFR exon 21 (23)	281 nt	17163-SP0449-L21566	B1	c.2573T>G=p.L858R

Note. The EGFR exon numbering has changed. From description version v02 onwards, we have adopted the NCBI exon numbering that is present in the NM_055228.3 sequence for EGFR gene, which is similar to the LRG exon numbering (LRG_304). This exon numbering used here may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets. Notify us of any mistakes: info@mlpa.com.

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Implemented Changes – compared to the previous SD006 product description versions

Version S01-03 – 10 April 2019 (15)
- Product description updated to a new template.

Version 02 – 5 October 2016 (14)
- Exon numbering of the EGFR gene has been changed and a note about the change is added in Table 1.
- Lot number removed throughout document.
- Explanation on when to include Binning DNA in the experiment adjusted on page 1.
- Minor textual and layout changes.

Version 01 (07)
- Not applicable, new document.