Product Description
SALSA® Reference Selection & Binning DNA SD084-S01

Version S01

Catalogue number
- **SD084**: SALSA Reference Selection & Binning DNA, 6 reactions

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.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.mrcholland.com. Reference Selection & Binning DNA is not known to contain any harmful agents.

Safety data sheet
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.

Intended purpose
The SALSA Reference Selection & Binning DNA SD084 is an in vitro diagnostic (IVD)[1] or research use only (RUO) reagent to be used in combination with SALSA MLPA Probemix P460-A1 SMA (Silent) Carrier, a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software for the selection of suitable reference DNA samples and the processes of linking all probe signals to their identity by use of the probe lengths. SD084 contains the targets of all probes included in the above-listed probemix, including two polymorphism specific probes for g.27134T>G and g.27706-27707delAT present on *SMN1* intron 7 and exon 8 respectively.

We recommend the use of SD084 only for initial experiments on DNA samples from healthy individuals with the intention of selecting suitable reference DNA samples. Reference Selection & Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of polymorphism signal(s).

Reference DNA samples for use in MLPA experiments should preferably be derived from the same type of tissue, and be purified by the same method, as the DNA samples to be tested. For certain applications, the selection of suitable reference DNA samples is complicated. Suitable reference samples for use with the P460 probemix should be diploid for SMN, but not contain the g.27134T>G and g.27706-27707delAT polymorphisms.

[1]Please note that this Reference Selection & Binning DNA is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

Experimental set up
Reference Selection & Binning DNA SD084 is a hybrid product with dual functionality. Its first use is for the selection of suitable reference samples. To select suitable reference samples, include three reactions with SALSA Reference Selection & Binning DNA SD084 in the initial MLPA experiment.

Its second use is for determining the location of the signal that will be generated when the polymorphisms are present in a sample. Inclusion of one reaction for binning purposes in the initial MLPA experiment is essential as it can also aid in data binning of the peak pattern using Coffalyser.Net software (see section Data analysis). Furthermore, one reaction for binning purposes 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).

MLPA reactions for reference sample selection and binning purposes should be performed with 5 µl of SD084 each. Please note that the SD084 reaction used for binning purposes in the initial MLPA experiment can be one of the three reactions included for the selection of suitable reference DNA samples.

Data analysis
Coffalyser.Net software should be used for analysis of MLPA experiments. Coffalyser.Net software is freely available at www.mrcholland.com.

To select suitable reference samples, the three SD084 reactions should be set as reference samples during data analysis of an experiment that only contains samples derived from healthy individuals. Suitable reference samples will be those samples from healthy individuals that have no signal for the two polymorphism specific probes and a final probe ratio between 0.80 and 1.20 for all other probes included in the probemix. Suitable reference samples selected as described can subsequently be used as reference samples in experiments with patient samples.

For binning, when performing the fragment analysis step in Coffalyser.Net, select SD084 in the *bin smpl* – column. By selecting the SD084 sample as your binning sample, probes will be correctly identified in the peak pattern across all patient samples.

Reference Selection & Binning DNA content
SD084 consists of a mixture of human female genomic DNA purified from a selected cell line and a titrated amount of synthetic DNA that contains the target sequences recognised by the polymorphism specific probes present in the MLPA probemix version specified above and in Table 1.

The cell line has two copies of the *SMN1* and *SMN2* genes and the P460-A1 probemix contains two probes specific for *SMN1* and one probe specific for *SMN2* (for details, see Table 1).

The synthetic DNA included in SD084 contains partial sequences of the *SMN1* gene. These sequences include two different polymorphisms which will be detected by MLPA probes present in the probemix and will generate polymorphism specific signals for these probes (for details, see Table 1).

Please note that the synthetic DNA also contains the target sequence of the 105 nt chromosome Y specific control fragment. As a result, the 100 and 105 nt control fragments indicate the presence of two copies chromosome X and one copy chromosome Y.

Table 1. P460 SMA (Silent) Carrier probe targets in Reference Selection & Binning DNA SD084-S01

Probe length (nt)	Gene/Exon	Probe ID	Probemix version	Copy number	Polymorphism details
131	Reference	00797-L25925	A1	2	
136	Reference	18457-L23634	A1	2	
143	***SMN1*** / intron 7	S0938-L26163	A1	n.a.	g.27134T>G
148	***SMN1*** / exon 8	S0961-L25586	A1	n.a.	g.27706-27707delAT
154	***SMN1*** / exon 8	S0960-L25957	A1	2	
163	Reference	02291-L17086	A1	2	
172	Reference	02978-L17087	A1	2	
183	***SMN1*** / exon 7	14919-L17081	A1	2	
191	Reference	00559-L17088	A1	2	
200	Reference	00976-L17298	A1	2	
208	Reference	12490-L17096	A1	2	
228	Reference	14498-L17101	A1	2	
237	Reference	02334-L17301	A1	2	
245	Reference	14293-L17100	A1	2	
255	Reference	13128-L17099	A1	2	
264	Reference	07630-L17091	A1	2	
272	Reference	14361-L17098	A1	2	
282	***SMN2*** / exon 7	14921-L17083	A1	2	
292	Reference	18491-L23716	A1	2	
301	Reference	12783-L13918	A1	2	
311	Reference	06425-L17092	A1	2	
321	Reference	01042-L17093	A1	2	
331	Reference	01043-L17094	A1	2	

Note: Mutation nomenclature used here may differ from literature! The exon numbering used in this SD084-S01 Reference Selection & Binning DNA product description is the traditional exon numbering (exons 1, 2a, 2b, and 3-8). This exon numbering is different from the NCBI reference sequences for *SMN1* and *SMN2*. Please consult the corresponding probemix product description for more information about exon numbering and gene transcripts used.

More information: www.mrcholland.com; www.mrcholland.eu	
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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.

Implemented changes in the product description
Version S01-03 – 02 June 2022 (03) - Product description adapted to a new template. *Version S01-02 – 13 July 2021 (02)* - Name of P460 probemix changed from 'SMA' to 'SMA (Silent) Carrier'. *Version S01-01 – 25 February 2021 (02)* - Not applicable, new document.