Product Description
SALSA® Reference Selection DNA SD082-S01

Version S01

Catalogue number
- **SD082**: SALSA Reference Selection 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 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
SALSA Reference Selection DNA SD082 is an in vitro diagnostic (IVD)[1] or research use only (RUO) reagent to be used in combination with SALSA MLPA Probemix P008-C1 PMS2, SALSA MLPA Probemix P021-B1 SMA, SALSA MLPA Probemix P060-B2 SMA Carrier, a SALSA MLPA Reagent Kit and Coffalyser.Net™ analysis software for the selection of suitable reference samples.

We recommend the use of this Reference Selection DNA SD082 only for initial experiments on DNA samples from healthy individuals with the intention to select suitable reference samples. Reference Selection DNA should never be used as a reference sample in the MLPA data analysis. Reference 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 samples is complicated. Furthermore, the percentage of all DNA samples that are suitable for use as a reference sample is population dependent. For P008-C1 PMS2, ~25% of Caucasian samples are expected to be suitable as reference samples, with two copies of *PMS2*, two copies of *PMS2CL* and two copies each of both alleles for five different SNPs in *PMS2* and *PMS2CL*. Based on analysis of cell lines from the 1000 genome collection, we estimate that suitable reference samples for use with P021-B1 SMA, with two copies of *SMN1*, two copies of *SMN2*, two copies of *NAIP* and no *SMN1/2Δ7-8* copies, are expected in approximately 35% of Caucasian samples, 50% of Asian samples and 15-20% of African samples. For P060-B2 SMA Carrier, we estimate that suitable reference samples, with two copies of *SMN1* and two copies of *SMN2*, are expected in approximately 46% of Caucasian samples, 54% of Asian samples and 23% of African samples.

[1]Please note that this Reference Selection 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
MLPA reactions for reference selection purposes should be performed with 5 µl of Reference Selection DNA. Initial experiments for the selection of suitable reference samples should include three reactions with SALSA Reference Selection DNA SD082 as well as reactions on a minimum of 20 independent DNA samples from healthy individuals (possibly more for populations with high *SMN1/2* variability). As reference samples should be treated identically to patient samples, choose reference samples from your own sample collection. Patient samples should not be included in the experiment.

Data analysis

Coffalyser.Net software should be used for analysis of MLPA experiments. Coffalyser.Net software is freely available at www.mrcholland.com. When the SD082 reactions are set as *reference samples* in the data analysis and the candidate reference samples from healthy individuals are set as *samples*, suitable reference samples will be those samples with a final probe ratio of ~1 (but at least between 0.80 and 1.20) for all probes included in the probemix. Suitable reference samples selected as described can subsequently be used as reference samples in experiments with patient samples.

Reference Selection DNA content

SD082 consists of human genomic DNA purified from a selected cell line. This cell line has two copies of the *PMS2* gene and two copies of the *PMS2CL* pseudogene. In addition, it has two copies each of both alleles for five different SNPs that are present in the *PMS2* gene and the *PMS2CL* pseudogene. Probes for these 10 alleles are present in the P008-C1 probemix (for details, see Table 1).

The cell line also has two copies of the *SMN1* and *SMN2* genes, no copies of the *SMN1/2Δ7-8* variant and two copies of the *NAIP* gene. The P021-B1 probemix contains four probes that are specific for either *SMN1* **or** *SMN2* and that detect two copies of these genes in SD082. The P021-B1 probemix contains 17 probes that detect a sequence which is present in both *SMN1* **and** *SMN2* and that detect four copies in SD082 (for details, see Table 2). The P060-B2 probemix contains four probes that are specific for either *SMN1* **or** *SMN2* and that detect two copies of these genes in SD082 (for details, see Table 3).

Table 1. P008 PMS2 probe targets in Reference Selection DNA SD082-S01

Probe length (nt)	Gene/Exon	Probe ID	Probemix version	Copy number	Remarks
128	Reference	00797-L00093	C1	2	-
133	***PMS2* exon 11**	14452-L00900	C1	2	-
140	***PMS2* exon 9**	14448-L16160	C1	2	-
146	***PMS2* exon 1**	07935-L16148	C1	2	-
154	Reference	02417-L04306	C1	2	-
160	Reference	08583-L08584	C1	2	-
165	***PMS2*/*PMS2CL* exon 11**	14453-L16164	C1	2	SNP probe
171	***PMS2*/*PMS2CL* exon 11**	14453-L16165	C1	2	SNP probe
177	Reference	04359-L03779	C1	2	-
184	***PMS2* exon 2**	01176-L16620	C1	2	-
190	***PMS2*/*PMS2CL* exon 14**	15768-L18167	C1	4	detects *PMS2* and *PMS2CL*
196	Reference	07510-L07172	C1	2	-
202	***PMS2*/*PMS2CL* exon 14**	14458-L16176	C1	2	SNP probe
208	***PMS2*/*PMS2CL* exon 14**	14458-L16177	C1	2	SNP probe
214	***PMS2*/*PMS2CL* exon 13**	14456-L16511	C1	2	SNP probe
220	***PMS2*/*PMS2CL* exon 13**	14456-L16512	C1	2	SNP probe
226	Reference	07083-L06712	C1	2	-
232	***PMS2* exon 5**	14445-L16154	C1	2	-
238	***PMS2*/*PMS2CL* intron 12**	14455-L16168	C1	2	SNP probe
244	***PMS2*/*PMS2CL* intron 12**	14455-L16169	C1	2	SNP probe
250	***PMS2* exon 6**	01180-L16157	C1	2	-
261	***PMS2*/*PMS2CL* exon 13**	15767-L17448	C1	4	detects *PMS2* and *PMS2CL*
268	Reference	19040-L09299	C1	2	-
276	***PMS2* exon 7**	01181-L16158	C1	2	-
283	***PMS2*/*PMS2CL* exon 12**	15769-L17786	C1	4	detects *PMS2* and *PMS2CL*
292	Reference	11087-L11770	C1	2	-
299	***PMS2* exon 8**	01182-L16159	C1	2	-
310	***PMS2* exon 3**	19910-L26895	C1	2	-
319	***PMS2* exon 10**	01184-L00745	C1	2	-
328	Reference	08543-L08544	C1	2	-
338	***PMS2* exon 1**	07934-L16147	C1	2	-
349	***PMS2*/*PMS2CL* exon 15**	14460-L04046	C1	2	SNP probe
356	***PMS2*/*PMS2CL* exon 15**	14460-L16180	C1	2	SNP probe
364	***PMS2* exon 11**	14451-L16163	C1	2	-
373	Reference	02528-L01959	C1	2	-
382	***PMS2*/*PMS2CL* exon 14**	15293-L17051	C1	4	detects *PMS2* and *PMS2CL*
390	***PMS2* exon 3**	19915-L26898	C1	2	-
400	***PMS2* exon 2**	14441-L16150	C1	2	-
409	***PMS2*/*PMS2CL* exon 15**	01189-L00750	C1	4	detects *PMS2* and *PMS2CL*
418	***PMS2* exon 4**	19906-L26893	C1	2	-
427	Reference	06029-L05485	C1	2	-
436	***PMS2* exon 6**	14447-L16623	C1	2	-
445	***PMS2* exon 9**	14449-L16622	C1	2	-
454	***PMS2* exon 5**	14446-L16621	C1	2	-
463	***PMS2* exon 10**	14450-L16162	C1	2	-
472	Reference	15978-L18133	C1	2	-
483	Reference	08480-L08491	C1	2	-

Note: Please consult the corresponding probemix product description for more information about exon numbering and gene transcripts used.

Table 2. P021 SMA probe targets in Reference Selection DNA SD082-S01

Probe length (nt)	Gene/Exon	Probe ID	Probemix version	Copy number	Remarks
175	Reference	00808-L00638	B1	2	-
184	***SMN1*/*SMN2* exon 1**	21519-L30024	B1	4	detects *SMN1* and *SMN2*
193	***SMN1*/*SMN2* intron 6**	22121-L31133	B1	4	detects *SMN1* and *SMN2*
199	***SMN1*/*SMN2* exon 5**	21518-L30023	B1	4	detects *SMN1* and *SMN2*
211	Reference	18964-L24756	B1	2	-
221	***SMN1*/*SMN2* exon 2a**	21517-L30022	B1	4	detects *SMN1* and *SMN2*
229	***SMN1*/*SMN2* intron 7**	22122-L31134	B1	4	detects *SMN1* and *SMN2*
238	***NAIP* exon 5**	01259-L00811	B1	2	-
247	Reference	19086-L24973	B1	2	-
256	Reference	19625-L26284	B1	2	-
265	***SMN1*/*SMN2* intron 7**	22124-L31136	B1	4	detects *SMN1* and *SMN2*
274	***SMN1* exon 7**	21488-L30891	B1	2	-
281	***SMN2* exon 7**	21489-L30892	B1	2	-
288	***SMN1*/*SMN2* exon 3**	21516-L30893	B1	4	detects *SMN1* and *SMN2*
295	***SMN1* exon 8**	21490-L29983	B1	2	-
301	***SMN2* exon 8**	21491-L29984	B1	2	-
310	Reference	20763-L28665	B1	2	-
319	***SMN1*/*SMN2* exon 2b**	14132-L15557	B1	4	detects *SMN1* and *SMN2*
328	***SMN1*/*SMN2* exon 2b**	21514-L30019	B1	4	detects *SMN1* and *SMN2*
337	Reference	19746-L26529	B1	2	-
346	***SMN1*/*SMN2* exon 3**	21513-L30018	B1	4	detects *SMN1* and *SMN2*
355	Reference	19127-L25074	B1	2	-
364	***SMN1*/*SMN2* exon 8**	01814-L00807	B1	4	detects *SMN1* and *SMN2*
373	Reference	18296-L25750	B1	2	-
382	***SMN1*/*SMN2* exon 1**	22196-L31253	B1	4	detects *SMN1* and *SMN2*
391	***SMN1*/*SMN2* intron 7**	22123-L31135	B1	4	detects *SMN1* and *SMN2*
400	***SMN1*/*SMN2* exon 8**	22126-L31138	B1	4	detects *SMN1* and *SMN2*
409	***SMN1*/*SMN2* exon 4**	01816-L30922	B1	4	detects *SMN1* and *SMN2*
418	***SMN1*/*SMN2* exon 6**	22194-L31251	B1	4	detects *SMN1* and *SMN2*
427	***SMN1*/*SMN2* intron 7**	22125-L31137	B1	4	detects *SMN1* and *SMN2*
436	Reference	19646-L26317	B1	2	-
445	Reference	20431-L27913	B1	2	-

Note: The *SMN1* and *SMN2* exon numbering used in this SD082-S01 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 these genes. Please consult the corresponding probemix product description for more information about exon numbering and gene transcripts used.

Table 3. P060 SMA Carrier probe targets in Reference Selection DNA SD082-S01

Probe length (nt)	Gene/Exon	Probe ID	Probemix version	Copy number
154	Reference	02595-L17085	B2	2
163	Reference	02291-L17086	B2	2
172	Reference	02978-L17087	B2	2
183	***SMN1* exon 7**	14919-L17081	B2	2
191	Reference	00559-L17088	B2	2
200	Reference	00976-L17298	B2	2
208	Reference	12490-L17096	B2	2
218	***SMN1* exon 8**	14881-L17082	B2	2
228	Reference	14498-L17101	B2	2
237	Reference	02334-L17301	B2	2
245	Reference	14293-L17100	B2	2
255	Reference	13128-L17099	B2	2
264	Reference	07630-L17091	B2	2
272	Reference	14361-L17098	B2	2
282	***SMN2* exon 7**	14921-L17083	B2	2
292	Reference	00824-L17097	B2	2
301	***SMN2* exon 8**	14878-L17084	B2	2
311	Reference	06425-L17092	B2	2
321	Reference	01042-L17093	B2	2
331	Reference	01043-L17094	B2	2
342	Reference	13399-L17297	B2	2

Note: The *SMN1* and *SMN2* exon numbering used in this SD082-S01 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 these genes. Please consult the corresponding probemix product description for more information about exon numbering and gene transcripts used.

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IVD	EUROPE* CE ISRAEL
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 S01-05 – 03 November 2021 (03) - Product description rewritten and adapted to a new template. - Intended purpose updated. - Information about P008-C1 probemix added. - Information about *PMS2* and *PMS2CL* genes added.

Version S01-04 – 11 June 2021 (02)
- Intended use updated.
- Additional information on selection of reference samples added to sections 'Experimental set up' and 'Data analysis'.
- Updated note under Table 1 and 2 to provide information on exon numbering of the *SMN1* and *SMN2* genes.
- UK added to the list of European countries that accept the CE-mark.

Version S01-03 – 26 June 2020 (02)
- Israel was added to the list of countries where this product is registered as IVD.

Version S01-02 – 29 April 2019 (02)
- Product is now registered for IVD use with P060.
- Table 2 added.

Version S01-01 – 06 November 2018 (01)
- Not applicable, new document.