Product Description SALSA® Binning DNA SD071-S01

Version S01.

Catalogue number: SD071: SALSA® Binning DNA, six reactions

To be used with the following SALSA MLPA probemixes: P247-A2 Chemokine-2 and P280-B3 SLC26A4, 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 SD071 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. 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 SD071 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 SD071 in the *bin smpl* –column. By selecting the SD071 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 SD071 DNA contains partial sequences of CCR2, CCR5, CX3CR1, HBA2 and SLC26A4 genes. These sequences include seven 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 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 one year is guaranteed, also after opening. The expiry date is mentioned on the label of the vial.

Table 1. Mutation-specific probe targets in SD071-S01 Binning DNA

Probemix	Gene/Exon	Probe length	Probe ID	Present in probemix version	Details
P247	CX3CR1 exon 2*	227 nt	12221-L13173	A2	c.841G>A; p.Val281Ile
	CX3CR1 exon 2*	265 nt	12225-L13174	A2	c.935C>T; .Thr312Met
	CCR2 exon 2	319 nt	12229-SP0083-L13172	A2	c.190G>A; p.Val64Ile
	CCR5 exon 3	251 nt	12226-L13154	A2	c.554_585del; c.Ser185Ilefs*32 (Delta-32 deletion)
P280	SLC26A4 exon 6	382 nt	09246-L13230	B3	c.707T>C; p.Leu236Pro
	SLC26A4 exon 8	319 nt	09249-L09440	B3	c.1001+1G>A (IVS8+1G>A)
	SLC26A4 exon 10	227 nt	20624-L28511	B3	c.1246A>C; p.Thr416Pro

*As these probes target common CX3CR1 mutations they give a small signal on the female genomic DNA pool from healthy individuals, which is included in SD071. Consequently, after adding the plasmid DNA containing the mutation-specific target sequences, the signal generated by these probes might be higher than the signal obtained on patients that contain a heterozygous mutation. Please consult product description of MLPA probemix P247-A2 for more information about these mutation-specific probes and the mutations targeted. Based on current NM_001171174.1 transcript of CX3CR1, codons 249 and 280 are numbered as 281 and 312 respectively, however in the probemix-specific product description of P247, nomenclature is according to OMIM and published literature (Val249Ile and Thr280Met).

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	
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Implemented Changes – compared to the previous SD071 product description versions

Version S01-02- 20 April 2018 (15)
  - Product description adapted to new probemix version in text on page 1 and in Table 1.
  - Minor textual adjustments done.
Version S01-01- 30 May 2017 (15)
  - Not applicable, new document.