Product Description SALSA® MLPA® Probemix P268-A3 DYSF

To be used with the MLPA General Protocol.

Version A3. As compared to version A2, four reference probes have been replaced and two probe lengths have been adjusted. For complete product history see page 7.

Catalogue numbers:
- **P268-025R:** SALSA MLPA Probemix P268 DYSF, 25 reactions.
- **P268-050R:** SALSA MLPA Probemix P268 DYSF, 50 reactions.
- **P268-100R:** SALSA MLPA Probemix P268 DYSF, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the 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.

General information: The SALSA MLPA Probemix P268 DYSF is a **research use only (RUO)** assay for the detection of deletions or duplications in the *DYSF* gene, which is associated with limb-girdle muscular dystrophy type 2B (LGMD2B).

Limb-girdle muscular dystrophies (LGMD) are a group of phenotypically and genotypically heterogeneous disorders, characterised by progressive weakness and atrophy of the muscles of the pelvic and shoulder girdle. Mutations of the Dysferlin gene (*DYSF*) are the cause of LGMD2B or dysferlinopathy. Patients with LGMD2B have symmetrical and selective involvement of proximal limb-girdle muscles. The disease shows wide intra- and interfamilial clinical variability. The age at onset ranges from 2 to 40 years, but the disease usually first appears in the second or third decade of life, with the development of proximal weakness in the lower limbs. Mutations in *DYSF* result in a cascade of events leading eventually to muscular dystrophy. The precise underlying mechanisms have yet to be elucidated.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1303/.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering: The *DYSF* exon numbering used in the P268-A3 DYSF product description is the exon numbering from the LRG_845 sequence, which contains 58 exons. The *DYSF* exon numbering has changed. From description version A3-01 onwards, we have adopted the LRG sequence exon numbering. The exon numbering of the NM_003494.4 sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences, the NM_003494.4 contains 55 exons. The exon numbering used in previous versions of this product description can be found in between brackets in Table 2. The exon numbering and NM_ sequence used have been retrieved on 12/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P268-A3 DYSF contains 48 MLPA probes with amplification products between 130 and 481 nucleotides (nt). This includes 40 probes for the *DYSF* gene targeting 40 out of 58 exons present in the LRG sequence. In addition, eight reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, one chromosome X, and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of Limb-girdle muscular dystrophies. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *DYSF* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P268 DYSF.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***DYSF* mutation database:** https://databases.lovd.nl/shared/genes/DYSF. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *DYSF* exons 31 and 33 but not exon 32) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P268-A3 DYSF

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*DYSF*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136	**DYSF probe** 08805-L08865		**Exon 9**
142	**DYSF probe** 08822-L08882		**Exon 33**
148	**DYSF probe** 08810-L22495		**Exon 16**
154	**DYSF probe** 08825-L08885		**Exon 38**
160	Reference probe 09787-L10202	15q15	
166	**DYSF probe** 08830-L08890		**Exon 46**
172	**DYSF probe** 08833-L22505		**Exon 50**
178	**DYSF probe** 08814-L22287		**Exon 22**
183	**DYSF probe** 08819-L08879		**Exon 29**
190	**DYSF probe** 08838-L08898		**Exon 57**
195	**DYSF probe** 10658-L08875		**Exon 24**
202	**DYSF probe** 11979-L12802		**Exon 48**
208	**DYSF probe** 08821-L08881		**Exon 32**
214	**DYSF probe** 08806-L11237		**Exon 11**
220	**DYSF probe** 08823-L22497		**Exon 35**
226	**DYSF probe** 08807-L08867		**Exon 12**
232	**DYSF probe** 08826-L08886		**Exon 39**
238	**DYSF probe** 08811-L22498		**Exon 18**
247 *	Reference probe 10808-L11455	4q25	
253 ¥	**DYSF probe** 08828-L32040		**Exon 44**
259 ¥	**DYSF probe** 08812-L32041		**Exon 19**
265	**DYSF probe** 11975-L22499		**Exon 3**
274	**DYSF probe** 08818-L08878		**Exon 27**
283	**DYSF probe** 08834-L08894		**Exon 52**
292	**DYSF probe** 08820-L08880		**Exon 31**
300	**DYSF probe** 10662-L22500		**Exon 8**
310	**DYSF probe** 10659-L08897		**Exon 56**
319	**DYSF probe** 11976-L13321		**Exon 5**
328	**DYSF probe** 08824-L08884		**Exon 36**
335 *	Reference probe 15117-L29502	9q33	
346	**DYSF probe** 11973-L22501		**Exon 42**
352	**DYSF probe** 11974-L22502		**Exon 1**
359 *	Reference probe 10727-L26803	6p12	
364	**DYSF probe** 08831-L22504		**Exon 47**
373	**DYSF probe** 08809-L08869		**Exon 15**
382	**DYSF probe** 08835-L11241		**Exon 54**
391	**DYSF probe** 08813-L08873		**Exon 20**
400	**DYSF probe** 08836-L08896		**Exon 55**
409	**DYSF probe** 11977-L12801		**Exon 25**
420	**DYSF probe** 08839-L08899		**Exon 58**
427	Reference probe 08577-L08578	17q23	
436	**DYSF probe** 10660-L08889		**Exon 45**
444	**DYSF probe** 08808-L08868		**Exon 14**
454	**DYSF probe** 11978-L11242		**Exon 26**
463	**DYSF probe** 10661-L08863		**Exon 6**
472 *	Reference probe 21765-L31980	13q12	
481	Reference probe 03328-L02715	3q26	

a) See above section on exon numbering for more information.

* New in version A3.
¥ Changed in version A3. Minor alteration, no change in sequence detected.

Table 2. *DYSF* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*DYSF* exon[a]	Ligation site NM_003494.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*439-441 (Exon 1)*		
352	11974-L22502	Exon 1	290-289, reverse	GCTTAGAGCAGT-TGCTCTTAAAGG	27.0 kb
265	11975-L22499	Exon 3 (2)	541-540, reverse	GATGACTTTGGT-TCTCTTCTTCAC	22.4 kb
319	11976-L13321	Exon 5 (4)	764-763, reverse	TGGGCTGCTTCT-TGGTGTCCAGCA	8.6 kb
463	10661-L08863	Exon 6 (5)	854-853, reverse	GGGAGGGCTCCA-GAGGAGTAGGGG	1.9 kb
300	10662-L22500	Exon 8 (6)	919-920	AGGAAGACACAG-AGGACCAGGGAC	2.0 kb
136	08805-L08865	Exon 9 (7)	1226-1227	CCCACTCTTCAA-TGAGGTGGGAGA	1.3 kb
214	08806-L11237	Exon 11 (9)	1320-1321	TCTCTCAGGACA-GATGCTCTCCTC	3.2 kb
226	08807-L08867	Exon 12 (10)	1346-1347	TTGATTGCAGAT-GGACGTGGGCAC	6.1 kb
444	08808-L08868	Exon 14 (12)	1532-1533	GGAGGACATTGA-AAGCAACCTGCT	2.1 kb
373	08809-L08869	Exon 15 (13)	1654-1655	TGAAACAGATCT-TTGGCTTCGAGA	6.7 kb
148	08810-L22495	Exon 16 (14)	1738-1739	GCAGCAAGATCT-TGGAGAAGACGG	4.2 kb
238	08811-L22498	Exon 18 (16)	1877-1878	TACCACCTACCT-GAGTATGTCGAA	10.2 kb
259	08812-L32041	Exon 19 (17)	1949-1948, reverse	AGTCTGAGGCTT-TCGAAGGCTTGA	1.7 kb
391	08813-L08873	Exon 20 (18)	2030-2031	CAGTCCCAGAGA-GTTCACAGGCTT	2.0 kb
178	08814-L22287	Exon 22 (20)	2305-2306	ACTACGGGAACA-AGTTCGACATGA	2.8 kb
195	10658-L08875	Exon 24 (22)	7 nt before exon 24, reverse	GCTTCCTGTGGA-ATGGGCAGGCAA	5.8 kb
409	11977-L12801	Exon 25 (23)	2623-2624	GTGACATCCATG-AGACACCCTCTG	2.4 kb
454	11978-L11242	Exon 26 (24)	2919-2918, reverse	AGCTTCCCACAA-TTCTTGCCACAG	3.9 kb
274	08818-L08878	Exon 27 (25)	3047-3048	GTTCAACCAGTT-TGCTGAGGGGAA	1.8 kb
183	08819-L08879	Exon 29 (27)	3299-3300	GGAAGAGGTGTT-TGAGAACCAGAC	0.9 kb
292	08820-L08880	Exon 31 (29)	3608-3609	GGAAGCACTGAA-AAGGGTGAGCCA	3.5 kb
208	08821-L08881	Exon 32 (30)	3671-3672	TTTTGGCTGGAA-GTTCCACCTCGA	15.4 kb
142	08822-L08882	Exon 33 (31)	3836-3837	CGTCTCCACCTT-GAGCTTCGGTGT	9.0 kb
220	08823-L22497	Exon 35 (33)	4060-4061	TCTTCTACGAGA-TCGAGATCTTTG	2.1 kb
328	08824-L08884	Exon 36 (34)	4187-4188	ACCGAGTCTGGA-ACGGATGCCACG	2.0 kb
154	08825-L08885	Exon 38 (36)	4323-4324	GTGCAGGAGACA-TCAAGGATCCTG	8.5 kb
232	08826-L08886	Exon 39 (37)	4392-4391, reverse	GGAACCATGTAG-ATGTTGGCCTCC	2.1 kb
346	11973-L22501	Exon 42 (40)	4818-4819	CTCATCGACATT-GATGACAAGGAG	30.6 kb
253	08828-L32040	Exon 44 (41)	4876-4877	ATTGGTGGAGCA-AATTCTTTGCCT	12.2 kb
436	10660-L08889	Exon 45 (42)	4956-4955, reverse	TCCAGCTGTGTG-TCATAGACCTGC	2.8 kb
166	08830-L08890	Exon 46 (43)	5199-5200	ATTGTCCGAGCA-TTTGGCCTGCAG	1.6 kb
364	08831-L22504	Exon 47 (44)	5273-5274	GAAGAAATCAGT-GAGTGACCAGGA	3.8 kb
202	11979-L12802	Exon 48 (45)	5440-5441	TCGACCTGGAGA-ACAGGCTGCTGT	3.0 kb
172	08833-L22505	Exon 50 (47)	22 nt before exon 50	CTCCTGCAACTT-TTTTGTCTTCTC	1.8 kb
283	08834-L08894	Exon 52 (49)	5894-5895	TATTATCTGGAA-TACCAGAGATGT	5.1 kb
382	08835-L11241	Exon 54 (51)	6140-6141	GACTGAGAGCAA-AATCCCAGCACG	4.9 kb
400	08836-L08896	Exon 55 (52)	6248-6247, reverse	TCTTGGCTGTCT-TGGCTGGCTTGG	1.9 kb
310	10659-L08897	Exon 56 (53)	6409-6410	TGACCTTGGAGA-TTGTAGCAGAGA	1.6 kb
190	08838-L08898	Exon 57 (54)	6613-6614	TCCTGCTGCTGT-TCCTGGCCATCT	3.8 kb
420	08839-L08899	Exon 58 (55)	6662-6663	TGCCATGAAGCT-GGTGAAGCCCTT	
		stop codon	*6679-6681 (Exon 58)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

P061 Lissencephaly	Contains probes for the *POMT1* and *POMGNT1* genes involved in LGMD2K and LGMD2O, respectively.
P116 SGCA	Contains probes for the *SGCA*, *SGCB*, *SGCD*, *SGCG* and *FKRP* genes involved in LGMD2D, 2E, 2F, 2C, and 2I, respectively.
P176 CAPN3	Contains probes for the *CAPN3* gene, involved in LGMD2A.
P048 LMNA/MYOT/ZMPSTE24	Contains probes for the *LMNA*, *MYOT*, *ZMPSTE24* and *CAV3* genes.
P436 ANO5	Contains probe for the *ANO5* gene, involved in LGMD2L.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P268 DYSF

- Bennett RR et al. (2009). Automated DNA mutation detection using universal conditions direct sequencing: application to ten muscular dystrophy genes. *BMC Genet*. 10:66.
- Ten Dam L et al. (2019). Autosomal recessive limb-girdle and Miyoshi muscular dystrophies in the Netherlands: The clinical and molecular spectrum of 244 patients. *Clin Genet*. 96(2):126-133.
- Izumi R et al. (2015). Genetic profile for suspected dysferlinopathy identified by targeted next-generation sequencing. *Neurol Genet*. 1(4):e36.
- Jin SQ et al. (2016). Dysferlin gene mutation spectrum in a large cohort of Chinese patients with dysferlinopathy. *Chin Med J (Engl).* 129(19):2287-2293.
- Meznaric M et al. (2011) Abnormal expression of dysferlin in skeletal muscle and monocytes supports primary dysferlinopathy in patients with one mutated allele. *EJoN.* 18(7): 1021-3.
- Walter MC et al. (2013) Treatment of dysferlinopathy with deflazacort: a double-blind, placebo-controlled clinical trial. *Orphanet J Rare Dis.* 8(1):26.

P268 Product history	
Version	*Modification*
A3	Four reference probes have been replaced and two probe lengths have been adjusted.
A2	Two reference probes have been replaced and one reference probe has been added. In addition, the control fragments have been replaced (QDX2).
A1	First release.

Implemented changes in the product description

Version A3-01 — 16 December 2019 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *DYSF* gene updated according to new version of the NM_ reference sequence.
- Exon numbering of the *DYSF* gene has been changed.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 11 – 7 September 2017 (55)
- Warning added in Table 1, 359 nt probe 08138-L22503.

Version 10 – 05 February 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- DYSF exon numbering adjusted.

- Manufacturer's address adjusted.
- Updated link for "Database of Genomic Variants".
- "Peak area" replaced with "peak height".

Version 09 (48)

- Electropherogram picture of old buffer (introduced Dec. 2012) removed.

More information: www.mlpa.com; www.mlpa.eu	
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