Product Description
SALSA® MLPA® Probemix P199-B3 HEXA

To be used with the MLPA General Protocol.

Version B3
For complete product history see page 7.

Catalogue numbers:
- **P199-025R:** SALSA MLPA Probemix P199 HEXA, 25 reactions.
- **P199-050R:** SALSA MLPA Probemix P199 HEXA, 50 reactions.
- **P199-100R:** SALSA MLPA Probemix P199 HEXA, 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.mrcholland.com).

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 MLPA General Protocol before use: www.mrcholland.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 P199 HEXA is a **research use only (RUO)** assay for the detection of deletions or duplications in the *HEXA* gene, which is associated with Tay-Sachs disease. This probemix can also be used to detect the presence of the c.1278insTATC (also known as c.1274_1277dupTATC (p.Tyr427Ilefs) and 1277TATC) and IVS12+1G>C (also known as c.1421+1G>C) mutations.

Tay-Sachs disease is an autosomal recessive, progressive neurodegenerative disorder, which is in the classic infantile form usually fatal before the age of two or three years. Defects in the *HEXA* gene encoding the alpha subunit of the hexosaminidase enzyme are the cause of Tay-Sachs disease. Some less severe defects in the *HEXA* gene cause a late-onset form of the disease. Due to some founder mutations, Tay-Sachs disease is frequent among Ashkenazi Jews. The c.1278insTATC mutation is found in 80% of the carriers of Tay-Sachs disease from the Ashkenazi Jew population. A 7.6-kb genomic deletion of the *HEXA* promoter and exon 1 is the major mutation found in Tay-Sachs disease carriers from the French-Canadian population.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1218/.

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 *HEXA* exon numbering used in this P199-B3 HEXA product description is the exon numbering from the NG_009017.2 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P199-B3 HEXA contains 28 MLPA probes with amplification products between 142 and 364 nucleotides (nt). This includes 18 probes for the *HEXA* gene, among which two probes specific for the *HEXA* c.1278insTATC and IVS12+1G>C mutations are present. These mutation specific probes will only generate a signal when the mutations are present. In addition, ten 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.mrcholland.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, and 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.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.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 different unrelated individuals who are from families without a history of Tay-Sachs disease. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

SALSA Binning DNA SD030
The SD030 Binning DNA provided with this probemix can be used for binning of all probes including the two mutation-specific probes (*HEXA* probe 06722-L06309 for the c.1278insTATC mutation and *HEXA* probe 06724-L06312 for the IVS12+1G>C mutation). SD030 Binning DNA is a mixture of genomic DNA from healthy

individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD030 Binning DNA in initial MLPA experiments is essential as it can be used to 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 capillaries have been renewed). 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). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD030 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

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.mrcholland.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 final ratio (FR) 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 FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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.
- 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: a lower injection voltage or a shorter injection time, 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 *HEXA* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P199 HEXA.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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.

***HEXA* mutation database**

https://databases.lovd.nl/shared/genes/HEXA. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 SNVs and unusual results (e.g., a duplication of *HEXA* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P199-B3 HEXA

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*HEXA*
64-105	Control fragments – see table in probemix content section for more information		
142	Reference probe 07721-L07431	7p	
148 ¥	**HEXA probe** 06713-L31539		**Exon 3**
155 ¥	**HEXA probe** 06718-L31540		**Exon 8**
160	Reference probe 09267-L09527	10q	
166 §	**HEXA probe** 06722-L06309		**c.1278insTATC**
172 ∞	**HEXA probe** 06724-L06312		**IVS12+1G>C**
179	Reference probe 01963-L03341	20q	
184	**HEXA probe** 06726-L06715		**Exon 14**
190	**HEXA probe** 06716-L06302		**Exon 6**
196	Reference probe 03547-L02913	11p	
204 ¥	**HEXA probe** 06710-L31546		**Exon 1**
211	**HEXA probe** 06714-L06300		**Exon 4**
220	Reference probe 00582-L00147	13q	
227	**HEXA probe** 15149-L16923		**Exon 7**
238	**HEXA probe** 06712-L06298		**Exon 2**
247	Reference probe 04108-L03468	9q	
257	**HEXA probe** 06720-L06306		**Exon 10**
265	**HEXA probe** 06715-L06717		**Exon 5**
275	Reference probe 15112-L27337	1p	
284	**HEXA probe** 06725-L06313		**Exon 13**
301	**HEXA probe** 06721-L06307		**Exon 11**
310	**HEXA probe** 06719-L06305		**Exon 9**
317	Reference probe 09906-L10319	16p	
327	**HEXA probe** 06723-L06310		**Exon 12**
336	**HEXA probe** 15150-L16924		**Exon 7**
346	Reference probe 05273-L04655	2p	
355	**HEXA probe** 06711-L06315		**Exon 1**
364 *	Reference probe 19252-L25357	16q	

[a] See section Exon numbering on page 1 for more information.

* New in version B3.

¥ Changed in version B3. Minor alteration, no change in sequence detected.

§ Mutation-specific probe. This probe will only generate a signal when the c.1278insTATC mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

∞ Mutation-specific probe. This probe will only generate a signal when the IVS12+1G>C mutation is present. It has been tested on artificial test DNA **but not on positive human samples!**

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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

Length (nt)	SALSA MLPA probe	*HEXA* exon[a]	Ligation site NM_000520.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*43-45 (Exon 1)*		
204	06710-L31546	Exon 1	172-173	ACCCGAACAACT-TTCAATTCCAGT	0.3 kb
355	06711-L06315	Exon 1	124 nt after exon 1	AGGCACTCCACT-TCCTCCTCGAGC	19.0 kb
238	06712-L06298	Exon 2	329-330	GAATGTGTTGGT-TGTCTCTGTAGT	1.0 kb
148	06713-L31539	Exon 3	3 nt after exon 3	CTCTCCGAGGTA-ACAAATTGGGGC	1.8 kb
211	06714-L06300	Exon 4	479-480	TAGCCAGCTTGT-TTGGAAATCTGC	0.6 kb
265	06715-L06717	Exon 5	594-595	CTGCCACTCTCT-AGCATCCTGGAC	1.9 kb
190	06716-L06302	Exon 6	669-670	GTAGATGATCCT-TCCTTCCCATAT	0.6 kb
227	15149-L16923	Exon 7	803-802 *reverse*	CAAACTCTGCAA-GCACACGGATAC	0.1 kb
336	15150-L16924	Exon 7	24 nt after exon 7	TCTGGGACCAGA-GGGACTCTGCTT	1.4 kb
155	06718-L31540	Exon 8	984-985	GTCTTCCCAGAT-TTTTATCTTCAT	1.0 kb
310	06719-L06305	Exon 9	1087-1088	GTGAGGACTTCA-AGCAGCTGGAGT	0.3 kb
257	06720-L06306	Exon 10	1138-1139	TCGTCTCTTCTT-ATGGCAAGGGCT	1.1 kb
301	06721-L06307	Exon 11	1236-1237	GATATTCCAGTG-AACTATATGAAG	0.1 kb
166 §	06722-L06309	Exon 11	1315-1316	GAACCGTATATC-TATCCTATGGCC	0.3 kb
327	06723-L06310	Exon 12	1417-1416 *reverse*	TTCTCCCCACAT-ACAAGCCTCTCC	0.1 kb
172 ∞	06724-L06312	Exon 12	1 nt after exon 12	CCCAGGCTCTGC-TAAGGGTTTTCG	0.7 kb
284	06725-L06313	Exon 13	1516-1517	AGTTGACATCTG-ACCTGACATTTG	1.7 kb
184	06726-L06715	Exon 14	1957-1958	GCCTTTGTGCTG-TTCTGCCTTGCC	
		stop codon	*1630-1632 (Exon 14)*		

[a] See section Exon numbering on page 1 for more information.

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

§ Mutation-specific probe. This probe will only generate a signal when the c.1278insTATC mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

∞ Mutation-specific probe. This probe will only generate a signal when the IVS12+1G>C mutation is present. It has been tested on artificial test DNA **but not on positive human samples!**

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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 P199 HEXA

- Georgiou T et al. (2014). The first family with Tay-Sachs disease in Cyprus: genetic analysis reveals a nonsense (c. 78G>A) and a silent (c. 1305C>T) mutation and allows preimplantation genetic diagnosis. *Meta Gene*. 2:200-205.
- Sheth J et al. (2018). Identification of deletion-duplication in HEXA gene in five children with Tay-Sachs disease from India. *BMC Med Genet*. 19:109.

P199 product history	
Version	*Modification*
B3	One reference probe has been removed and one reference probe has been replaced, in addition three probe lengths have been adjusted.
B2	Two reference probes have been replaced and the control fragments adjusted (QDX2).
B1	Two HEXA probes and two reference probes have been replaced. In addition, four extra control fragments at 88-96-100-105 nt have been included.
A1	First release.

Implemented changes in the product description
Version B3-02– 20 September 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *HEXA* gene updated according to new version of the NM_ reference sequence. - Minor textual changes. Version B3-01 – 12 February 2019 (01P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2, new picture included).

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