Product Description
SALSA® MLPA® Probemix P164-B3 IDS

To be used with the MLPA General Protocol.

Version B3
For complete product history see page 7.

Catalogue numbers:
- **P164-025R:** SALSA MLPA Probemix P164 IDS, 25 reactions.
- **P164-050R:** SALSA MLPA Probemix P164 IDS, 50 reactions.
- **P164-100R:** SALSA MLPA Probemix P164 IDS, 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 P164 IDS is a **research use only (RUO)** assay for the detection of deletions or duplications in the *IDS* gene, which is associated with Hunter syndrome.

Defects in the Iduronate 2-sulfatase (*IDS*) gene on chromosome X are the cause of mucopolysaccharidosis type II (MPS II) or Hunter syndrome. Partial deletions or complete *IDS* gene deletions are estimated to be the cause of disease in 20% of all cases. Patients with full deletions or gross rearrangements of the *IDS* gene have in general a severe clinical presentation. However, most individuals with Hunter syndrome have point mutations in the *IDS* gene, none of which will be detected by the MLPA technique.

A pseudogene of *IDS* gene (*IDSP1*) is located at short distance of the real gene. A recombination event between the *IDS* gene and the *IDSP1* pseudogene is the cause of disease in an estimated 13% of the Hunter syndrome patients. In most patients this recombination results in an inversion. It is probably not possible to detect most inversions with this MLPA assay. In view of the close distance and high similarity between the *IDS* and *IDSP1* genes, gene conversion might occur and great care should be taken with interpretation of results obtained with this P164-B3 probemix.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1274/.

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 *IDS* exon numbering used in this P164-B3 IDS product description is the exon numbering from the NG_011900.3 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 P164-B3 IDS contains 24 MLPA probes with amplification products between 136 and 373 nucleotides (nt). This includes eleven probes for the *IDS* gene, and also includes five flanking probes, with three probes targeting the upstream region of the *IDS* gene and two probes the downstream region of the *IDSP1* pseudogene. 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.mrcholland.com).

This probemix contains ten 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 two chromosome Y-specific fragments (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)
105-118	Y-fragments (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 of the same sex 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 Hunter syndrome. It is recommended to use samples of the same sex to facilitate interpretation. 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.

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:

Copy Number status: Male samples	Final ratio
Normal	0.80 < FR < 1.20
Deletion	FR = 0
Duplication	1.65 < FR < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	Final ratio
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 *IDS* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P164 IDS.
- 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.

***IDS* mutation database**

https://databases.lovd.nl/shared/genes/IDS. 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 SNVs and unusual results (e.g., a duplication of *IDS* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P164-B3 IDS

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*IDS*	Flanking
64-118	Control fragments – see table in probemix content section for more information			
136	Reference probe 02629-L03719	Xq13		
148	**IDS probe** 05238-L04618		**Exon 1**	
166 ¬«	**FMR1 probe** 02927-L03721			1.6 Mb centromeric of *IDS*
184	**IDS probe** 05246-L04626		**Exon 7**	
192	Reference probe 13091-L14310	Xq21		
198	Reference probe 05882-L17677	Xq22		
205	**IDS probe** 05240-L17080		**Exon 1**	
212	**IDS probe** 05247-L04627		**Exon 8**	
220	Reference probe 06107-L05562	Xq25		
229	**IDS probe** 05241-L04621		**Exon 2**	
238	**IDS probe** 14918-L16651		**Exon 8**	
247	Reference probe 07866-L07677	Xp22		
256 «	**IDS probe** 15036-L16785		**Exon 3**	
265	**IDS probe** 05249-L04629		**Exon 9**	
276	**IDS probe** 05243-L04623		**Exon 4**	
292 ¬	**IDSP1 probe** 05250-L04630			0.5 kb downstream of *IDSP1*
301	Reference probe 02635-L02102	Xp11		
310	**IDS probe** 05244-L04624		**Exon 5**	
319 ¬	**IDSP1 probe** 05251-L04631			1.9 kb downstream of *IDSP1*
328	**IDS probe** 07284-L06908		**Exon 6**	
337 ¬	**AFF2 probe** 12914-L03200			0.5 Mb centromeric of *IDS*
346	Reference probe 05125-L04515	Xq26		
355 ¬	**AFF2 probe** 03741-L03201			0.5 Mb centromeric of *IDS*
373	Reference probe 01282-L00965	Xq22		

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

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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. *IDS* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*IDS* exon[a]	Ligation site NM_000202.8	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
319 ¬	05251-L04631	1.9 kb down-stream of *IDSP1*		CTTGGAGGAAAT-GAGTGAAAAGAT	1.4 kb
292 ¬	05250-L04630	0.5 kb down-stream of *IDSP1*		TCATCCTTCCCT-TGGCCTACCTGT	21.1 kb
		start codon	*170-172 (Exon 1)*		
148	05238-L04618	Exon 1	74 nt before exon 1	TCGGCCACGCCT-ATTGCTGCAGGA	0.2 kb
205	05240-L17080	Exon 1	78-79	GGTTCCCGACGA-GGAGGTCTCTGT	1.0 kb
229	05241-L04621	Exon 2	304-305	ATCATCGTGGAT-GACCTGCGCCCC	1.1 kb
256 « #	15036-L16785	Exon 3	145 nt after exon 3, reverse	GTTGACAAGTCA-GTTTTTTAGAAA	2.2 kb
276	05243-L04623	Exon 4	640-641	TCTTTTCCACCT-TATCATCCTTCC	2.8 kb
310	05244-L04624	Exon 5	804-805	GTTGGAAAAGAT-GAAAACGTCAGC	1.7 kb
328	07284-L06908	Exon 6	905-906	ATCCCTTGGAGA-ACATCACCCTGG	6.1 kb
184	05246-L04626	Exon 7	1137-1138	CGATCTTCAGCT-GGCCAACAGCAC	3.1 kb
212 +	05247-L04627	Exon 8	157 nt before exon 8	AGTTGAAATAAC-CCTTTCTGTGGT	0.3 kb
238	14918-L16651	Exon 8	1306-1307	AAGCTTTTCCCT-TACCTCGACCCT	4.2 kb
265	05249-L04629	Exon 9	1838-1839	TGCCAACCATGG-ATGGCAAATGTG	597.0 kb
		stop codon	*1820-1822 (Exon 9)*		
355 ¬	03741-L03201	*AFF2* exon 8		AGTGATGAAGAT-GACCTTGAGCCT	42.5 kb
337 ¬	12914-L03200	*AFF2* exon 7		TTGAAAGACACT-GACTTTGTAGAA	910.7 kb
166 « ¬	02927-L03721	*FMR1* exon 9		AAAAGCTAGAAG-CTTTCTCGAATT	

[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.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
+ This sequence is present in exon 8 of the alternative transcript NM_006123.5 (ligation site at position 1233-1234), which represents transcript variant 2 of the IDS gene.

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 P164 IDS

- Agrawal N et al (2022). Genotype-phenotype spectrum of 130 unrelated Indian families with Mucopolysaccharidosis type II. *Eur J Med Genet*, 65(3), 104447.
- Deribessa SJ et al. (2021). A 1-year and 4-month-old child with mucopolysaccharidoses type II: A clinical case report from Ethiopia. *Clin Case Rep*, 9(11), e05122.
- Fang X et al. (2022). Molecular analysis and novel variation identification of Chinese pedigrees with mucopolysaccharidosis using targeted next-generation sequencing. *Clin Chim Acta*, 524, 194-200.
- Galvis J et al. (2014). Deep genotyping of the IDS gene in Colombian patients with Hunter syndrome. In JIMD Rep, Volume 19 (pp. 101-109). *Springer*, Berlin, Heidelberg.
- Gonçalves NJN et al. (2022). Comparative assessment of cytogenetic and molecular approaches for product of conception: a retrospective study of 726 cases. *Fertil Steril*, 118(4), e179.
- Oshima J et al. (2011). LCR-initiated rearrangements at the IDS locus, completed with Alu-mediated recombination or non-homologous end joining. *J Hum Genet*, 56(7), 516.
- Ramírez-Hernández MA et al. (2022). Mutational spec-trum of the iduronate-2-sulfatase gene in Mexican patients with Hunter syndrome. *Eur Rev Med Pharmacol*. Sci, 26, 5115-5127.
- Uttarilli A et al. (2016). Identification and characterization of 20 novel pathogenic variants in 60 unrelated Indian patients with mucopolysaccharidoses type I and type II. *Clin Genet*, 90(6), 496-508.
- Vollebregt A et al. (2017). Genotype–phenotype relationship in mucopolysaccharidosis II: predictive power of IDS variants for the neuronopathic phenotype. *Dev Med Child Neurol*, 59(10), 1063-1070.

P164 product history	
Version	*Modification*
B3	The Y control fragment on 116 nt has been elongated to 118 nt.
B2	Compared to previous version, one reference probe has been replaced and control fragments adjusted (QDX2).
B1	Eight probes, including two IDS probes have been replaced and one IDS probe has been removed. In addition, four extra control fragments have been added.
A1	First release.

Implemented changes in the product description

Version B3-02 – 09 March 2023 (04P)
- Product description rewritten and adapted to a new template.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version B3-01 — 19 September 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).
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Ligation sites of the probes targeting the *IDS* gene updated according to new version of the NM_ reference sequence.

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