Product Description
SALSA® MLPA® Probemix P457-A3 DHCR7

To be used with the MLPA General Protocol.

Version A3
As compared to version A2, two reference probes have been replaced. For the complete product history see page 6.

Catalogue numbers:
- **P457-025R:** SALSA MLPA Probemix P457 DHCR7, 25 reactions.
- **P457-050R:** SALSA MLPA Probemix P457 DHCR7, 50 reactions.
- **P457-100R:** SALSA MLPA Probemix P457 DHCR7, 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 P457 DHCR7 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *DHCR7* gene, which is associated with the Smith-Lemli-Opitz syndrome (SLOS).

SLOS is an autosomal recessive syndrome, phenotypically characterized by intellectual disability, facial dysmorphism, syndactyly of second and third toes, and holoprosencephaly. Defects in the *DHCR7* gene, localised on chromosome 11q13, are the main cause of SLOS (Jira et al. 2003). The protein encoded by this gene is 7-dehydrocholesterol reductase, which plays a catalyser role in the final step of cholesterol biosynthesis. Therefore, the defects in the *DHCR7* gene result in an abnormality in cholesterol metabolism. These defects are in its majority point mutations, although a few deletions have also been identified to cause SLOS (Waterham et al. 2012). The incidence of SLOS is of about 1:55,000 in North America, while in Central Europe it is significantly higher (~ 1:15,000) (Nowaczyk et al. 2006).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1143/.

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 *DHCR7* exon numbering used in this P457-A3 DHCR7 product description is the exon numbering from the LRG_340 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 LRG 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 P457-A3 DHCR7 contains 23 MLPA probes with amplification products between 148 and 318 nucleotides (nt). This includes eleven probes for the *DHCR7* gene. In addition, 12 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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 SLOS. 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 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 *DHCR7* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P457 DHCR7.
- 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.

***DHCR7* mutation database**

https://databases.lovd.nl/shared/genes/DHCR7. 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 *DHCR7* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P457-A3 DHCR7

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*DHCR7*
64-105	Control fragments – see table in probemix content section for more information		
148	Reference probe 03267-L02704	3q	
154	**DHCR7 probe** 20162-L27426		**Exon 3**
160 *	Reference probe 12741-L13835	21q	
166	**DHCR7 probe** 20163-L27427		**Exon 9**
172	**DHCR7 probe** 20164-L27428		**Exon 4**
178	Reference probe 02865-L02617	17q	
184	Reference probe 17862-L22121	19q	
190	**DHCR7 probe** 20165-L27429		**Exon 8**
202	**DHCR7 probe** 20166-L27430		**Exon 1**
208	Reference probe 13384-L25019	6q	
220	**DHCR7 probe** 20167-L27431		**Exon 9**
226	Reference probe 18912-L24507	1p	
232	Reference probe 10410-L12202	9q	

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*DHCR7*
238	**DHCR7 probe** 20168-L27432		**Exon 7**
247	Reference probe 11138-L16379	13q	
256	**DHCR7 probe** 20169-L27433		**Exon 2**
266	Reference probe 15653-L18125	5q	
274	**DHCR7 probe** 20170-L27434		**Exon 6**
283 *	Reference probe 03686-L03101	14q	
292	**DHCR7 probe** 20171-L27435		**Exon 9**
301	Reference probe 06749-L06353	8q	
310	**DHCR7 probe** 20172-L27436		**Exon 5**
318	Reference probe 16438-L23633	18q	

[a] See section Exon numbering

on page 1 for more information.

* New in version A3.

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. *DHCR7* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*DHCR7* exon[a]	Ligation site NM_001360.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*236-238 (Exon 3)*		
202	20166-L27430	Exon 1	42 nt before exon 1	CCTCCGATTGGC-AGGCAATCGCTG	0.7 kb
256	20169-L27433	Exon 2	106-107	TTCCTTGCAGGA-CTTTAGCCGGTT	2.9 kb
154	20162-L27426	Exon 3	30 nt after exon 3	CTCCCAGCTAGA-AAGTAAAGTGTG	0.8 kb
172	20164-L27428	Exon 4	491-492	TCTCGGACATCT-GGGCCAAGACTC	1.7 kb
310	20172-L27436	Exon 5	571-572	CTTCTGTACACG-TCTCTCCCTGAC	1.0 kb
274	20170-L27434	Exon 6	731-732	CTCATCTCCTGT-CCTGGTTCTCGC	2.5 kb
238	20168-L27432	Exon 7	1047-1048	CAATGCCATGGT-CCTGGTCAACGT	1.1 kb
190	20165-L27429	Exon 8	1173-1174	CGACTGTGTCTG-GCTGCCTTATCT	2.2 kb
292	20171-L27435	Exon 9	1415-1416	ACCACAGCAAGC-TGCTGGTGTCGG	0.5 kb
166	20163-L27427	Exon 9	1948-1947, reverse	TGTCTCCAAAGG-ACTAGTAAAGGT	0.7 kb
220	20167-L27431	Exon 9	2592-2593	CTCCGAGTAGAG-TTCATCTTTATA	
		stop codon	*1661-1663 (Exon 9)*		

[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.

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.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P187 Holoprosencephaly	Detection of holoprosencephaly (HPE). Contains probes for the *SHH*, *ZIC2*, *SIX3*, *TGIF1*, *TRAPPC10*, *GLI2*, *PTCH1* and *FBXW11* genes.

References

- Jira PE et al. (2003). Smith-Lemli-Opitz syndrome and the *DHCR7* gene. *Ann Hum Genet*. 67:269-280.
- Nowaczyk MJM et al. (2006). *DHCR7* mutation carrier rates and prevalence of the RSH/Smith-Lemli-Opitz syndrome: Where are the patients? *Am J Med Genet A*. 140A:2057-2062.
- 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.
- Waterham HR et al. (2012). Mutational spectrum of Smith-Lemli-Opitz syndrome. *Am J Med Genet C: Semin Med Genet*. 160C:263-284.

P457 product history	
Version	*Modification*
A3	Two reference probes have been replaced.
A2	Two reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version A3-03 –20 November 2024 (04P) - The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate. Version A3-02 – 30 July 2024 (04P) - Updated numbering of tables. Version A3-01– 08 September 2022 (04P) - 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). - Various minor textual or layout changes. - Ligation sites of the probes targeting the *DHCR7* gene updated according to new version of the NM_ reference sequence.

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