Product Description
SALSA® MLPA® Probemix P183-C1 EDA-EDAR-EDARADD

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 9.

Catalogue numbers:
- **P183-025R:** SALSA MLPA Probemix P183 EDA-EDAR-EDARADD, 25 reactions.
- **P183-050R:** SALSA MLPA Probemix P183 EDA-EDAR-EDARADD, 50 reactions.
- **P183-100R:** SALSA MLPA Probemix P183 EDA-EDAR-EDARADD, 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 P183 EDA-EDAR-EDARADD is a **research use only (RUO)** assay for the detection of deletions or duplications in the genes *EDA*, *EDAR*, *EDARADD* and *WNT10A*, which are associated with hypohidrotic ectodermal dysplasia (HED).

X-linked HED (XLHED) is the most common form of ectodermal dysplasia (ED) in humans, with an incidence of approximately 1 per 100,000 births. This syndrome is characterised by absence or aberrant development of exocrine sweat glands, leading to heat intolerance with excessively dry skin, abnormal spiky or absent teeth and sparse hair on the scalp and body. These structures are affected together or in different combinations. It has been shown that mutations of the X-linked *EDA* gene are the major causative factor for this disorder. *EDA* encodes the ectodysplasin A (EDA) protein, which has been recognised as a member of the tumour necrosis factor (TNF) superfamily. *EDAR* encodes the ectodysplasin A receptor. Mutations in this gene result in autosomal dominant and recessive forms of hypohidrotic ectodermal dysplasia. *EDARADD* encodes the *EDAR*-associated death domain protein which is also involved in ectodermal dysplasia. *WNT10A* belongs to the WNT gene family, which encodes secreted signalling proteins.

The *EDA* (*ED1*) gene (8 exons) spans ~423 kb of genomic DNA and is located on Xq13.1, 69 Mb from the p-telomere. The *EDAR* gene (12 exons) spans ~95 kb of genomic DNA and is located on 2q13, 109 Mb from the p-telomere. The *EDARADD* gene (6 exons) spans ~91 kb of genomic DNA and is located on 1q43, 235 Mb from the p-telomere. The *WNT10A* gene (4 exons) spans ~13 kb of genomic DNA and is located on 2q35, 219 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1112/.

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 *EDA* exon numbering used in this P183-C1 EDA-EDAR-EDARADD product description is the exon numbering from the NG_009809.2 sequence. The *EDAR* exon numbering is the exon numbering from the NG_008257.1 sequence. The *EDARADD* exon numbering is the exon numbering from the NG_011566.1 sequence. The *WNT10A* exon numbering is the exon numbering from the NG_012179.1 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 P183-C1 EDA-EDAR-EDARADD contains 48 MLPA probes with amplification products between 130 and 503 nucleotides (nt). This includes 11 probes for the *EDA* gene (one for each exon present in transcript variant 1 (NM_001399.5), two probes for exons 1, 2 and 8), 12 probes for the *EDAR* gene (one for each exon), nine probes for the *EDARADD* gene (one for each exon present in transcript variant A (NM_145861.4), two probes for exons 1, 4 and 5), and four probes for the *WNT10A* gene (one for each exon). This probemix furthermore contains one flanking probe upstream of *EDA* and two additional flanking probes further away on the X chromosome. In addition, nine 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 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 ectodermal dysplasia. 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. Sample ID numbers from the Coriell Institute in the table below have been tested with this P183-C1 probemix at MRC Holland and can be used as a positive control samples to detect the copy number alterations shown in the table. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration	Altered target genes in P183-C1	Expected copy number alteration
NA05347	Coriell Institute	1q32.1-q44	*EDARADD*	Heterozygous duplication
NA01229	Coriell Institute	2q32.3-q37.3	*WNT10A*	Heterozygous duplication
NA10020	Coriell Institute	1q42.13-q43	*EDARADD*	Heterozygous deletion
NA10401	Coriell Institute	chr2	*EDAR* and *WNT10A*	Heterozygous duplication
NA10918	Coriell Institute	2q34-q36.1	*WNT10A*	Heterozygous deletion

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 when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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 *EDA*, *EDAR*, *EDARADD* and *WNT10A* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P183 EDA-EDAR-EDARADD.
- 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.

***EDA*, *EDAR*, *EDARADD* and *WNT10A* mutation databases**

https://databases.lovd.nl/shared/genes/EDA, https://databases.lovd.nl/shared/genes/EDAR, https://databases.lovd.nl/shared/genes/EDARADD and https://databases.lovd.nl/shared/genes/WNT10A. 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 *EDA* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P183-C1 EDA-EDAR-EDARADD

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*EDA*	*EDAR*	*EDARADD*	*WNT10A*
64-105	Control fragments – see table in probemix content section for more information					
130 *	Reference probe 13351-L14781	8q				
136 *	**WNT10A probe** 16457-L18910					**Exon 1**
143	**EDAR probe** 14199-L15813			**Exon 7**		
148	**EDARADD probe** 13478-L14940				**Exon 2**	
154	**EDAR probe** 13816-L15310			**Exon 5**		
160	Reference probe 07992-L07773	7q				
166	**EDA probe** 14200-L15814		**Exon 2**			
172	**EDARADD probe** 13481-L14943				**Exon 4**	
178	Reference probe 05976-L05401	20p				
190	**EDA probe** 08897-L08989		**Exon 6**			
196	**EDA probe** 06032-L05487		**Exon 1**			
202	**EDARADD probe** 13484-L15493				**Exon 6**	
211	**EDA probe** 06039-L05494		**Exon 7**			
219	**EDA probe** 06034-L05489		**Exon 2**			
226	**EDAR probe** 12781-L13916			**Exon 4**		
232 *	Reference probe 16429-L18882	18q				
238 ¬	GLA probe 05160-L04564		downstream			
248	**EDA probe** 06033-L05488		**Exon 1**			
256	**EDA probe** 06041-L05496		**Exon 8**			
262	**EDARADD probe** 14202-L16587				**Exon 1**	
267	**EDAR probe** 12782-L15494			**Exon 3**		
274	**EDARADD probe** 13479-L14941				**Exon 3**	
283	**EDA probe** 06035-L05490		**Exon 3**			
292	**EDA probe** 06037-L05492		**Exon 5**			
297 *	**WNT10A probe** 16460-L25670					**Exon 4**
303 ¥	**EDAR probe** 12783-L25671			**Exon 11**		
310 *	Reference probe 18380-L25673	10q				
317	**EDA probe** 06040-L05495		**Exon 8**			
328 *	Reference probe 09227-L09545	5q				
338	**EDAR probe** 12785-L15496			**Exon 1**		
346 ¬	ATP7A probe 03149-L14468		downstream			
355	**EDAR probe** 12786-L13921			**Exon 8**		
362	**EDARADD probe** 13482-L14944				**Exon 5**	
373 ¥ Ж	**EDA probe** 12784-SP0830-L26024		**Exon 4**			
382 *	**WNT10A probe** 16458-L18911					**Exon 2**
390 ¥	**EDARADD probe** 13477-L25672				**Exon 1**	
398 ¥ ¬	EFNB1 probe 03764-L25674		upstream			
409	**EDAR probe** 12787-L13922			**Exon 2**		
417	**EDAR probe** 13817-L15311			**Exon 10**		
425	**EDAR probe** 12789-L13924			**Exon 6**		
436 ¥	**EDAR probe** 12790-L19277			**Exon 12**		
449	**EDARADD probe** 13480-L14942				**Exon 4**	
454 *	Reference probe 02144-L01619	13q				
463	**EDARADD probe** 13483-L14945				**Exon 5**	
474 *	**WNT10A probe** 16459-L18912					**Exon 3**
481	**EDAR probe** 14203-L15817			**Exon 9**		
493 *	Reference probe 09772-L22978	15q				
503 *	Reference probe 06676-L23439	11p				

[a] See section Exon numbering on page 2 for more information.

* New in version C1.

¥ Changed in version C1. Minor alteration, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ 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. P183-C1 probes arranged according to chromosomal location

Table 2a. *EDA* gene

Length (nt)	SALSA MLPA probe	*EDA* exon[a]	Ligation site NM_001399.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
398 ¬	03764-L25674	*EFNB1* gene		ATCTGCCCCCGA-GCAGAAGCAGGG	777.5 kb
		start codon	*197-199 (Exon 1)*		
196	06032-L05487	Exon 1	104-105	CCCCCAGCCGAT-GGCAGGACAGTA	0.2 kb
248	06033-L05488	Exon 1	335-336	TCCTGGGTTTCT-TTGGCCTCTCGC	340.3 kb
166	14200-L15814	Exon 2	311 nt before exon 2	TATGTGTGGGAA-GTTAGAAATTTG	0.4 kb
219	06034-L05489	Exon 2	649-650	GAAGAAGAAAGT-AGGCGTGTTCGC	66.0 kb
283	06035-L05490	Exon 3	98 nt before exon 3	CTCCCTCAAATT-TGCAGTGTCTTG	4.9 kb
373 Ж	12784-SP0830-L26024	Exon 4	899-898; 872-871 *reverse*	GAACTCACCAGA-27 nt spanning oligo-AGGACCAGGAGG	1.5 kb
292	06037-L05492	Exon 5	913-914	GGTGCTGCTGAT-AAAGCTGGAACT	1.2 kb
190	08897-L08989	Exon 6	206 nt after exon 6	AAATTGCTTTAG-AATCACTGATGA	2.7 kb
211	06039-L05494	Exon 7	1032-1033	TCGCATCACTAT-GAACCCCAAGGT	2.0 kb
317	06040-L05495	Exon 8	1173-1174	GGTGGTGGATGA-GAAGCCCTTCCT	3.0 kb
256	06041-L05496	Exon 8	4205-4206	TGAATTTGCCAG-AGGACCAATGCT	7.8 Mb
		stop codon	*1370-1372 (Exon 8)*		
346 ¬	03149-L14468	*ATP7A* gene		TAACCATAGGAT-AGAGAAACCAGG	23.5 Mb
238 ¬	05160-L04564	*GLA* gene		GGGTAAAGGAGT-GGCCTGTAATCC	

Table 2b. *EDAR* gene

Length (nt)	SALSA MLPA probe	*EDAR* exon[a]	Ligation site NM_022336.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*280-282 (Exon 2)*		
338	12785-L15496	Exon 1	194-195	CCCAGAACTGGA-TGGTACCTGACT	58.0 kb
409	12787-L13922	Exon 2	8 nt before exon 2	TAATGACGCATG-TCTTTCAGGTGA	0.8 kb
267	12782-L15494	Exon 3	386-387	TGAGAACGAGTA-CTACAACCAGAC	0.8 kb
226	12781-L13916	Exon 4	39 nt before exon 4 *reverse*	CAGGTGAGTGCA-GCAGCCAAACCA	6.1 kb
154	13816-L15310	Exon 5	715-714 *reverse*	CTCACATTCCTT-GGTGTTGGGGGG	10.7 kb
425	12789-L13924	Exon 6	5 nt after exon 6	CACAAAGGTGAG-GAGGGTGCTCAG	1.6 kb
143	14199-L15813	Exon 7	5 nt before exon 7 *reverse*	GAGAGTTCTGTG-GGTGGAGAGAAG	0.3 kb
355	12786-L13921	Exon 8	23 nt after exon 8	AGGGCTTCCACA-CACCATGTGCAC	0.3 kb
481	14203-L15817	Exon 9	1079-1080	AAAGCCCACCAA-GAGGTATGTGGA	2.5 kb
417	13817-L15311	Exon 10	1196-1195 *reverse*	TGGCCAGGTGAA-CCAGCGACAGCA	1.6 kb
303	12783-L25671	Exon 11	1283-1282 *reverse*	CTCCACACACGT-TGGCATACACAT	9.5 kb
436	12790-L19277	Exon 12	1724-1725	AGAATCAAGGCT-TTTGTGATATGT	
		stop codon	*1624-1626 (Exon 12)*		

Table 2c. *EDARADD* gene

Length (nt)	SALSA MLPA probe	*EDARADD* exon[a]	Ligation site NM_145861.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*160-162 (Exon 1)*		
262	14202-L16587	Exon 1	154-153 *reverse*	GCCCATGGCGAT-GTTGAGTTTGGC	0.1 kb
390	13477-L25672	Exon 1	41 nt after exon 1	TTCTCAGAGCTG-AGTTTTTAGCCA	14.7 kb
148	13478-L14940	Exon 2	249-248 *reverse*	CTAGGGTCTGTG-TCTTCCACTGGT	5.0 kb
274	13479-L14941	Exon 3	303-304	CCCATTCAAGAT-ACGGAACTCCCT	13.1 kb
449	13480-L14942	Exon 4	350-351	TACTTTGAACTG-CCCACGAAATTC	0.2 kb
172	13481-L14943	Exon 4	132 nt after exon 4 *reverse*	ATGCTTAGAGAC-CATTTGCAAACC	40.5 kb
362	13482-L14944	Exon 5	162 nt before exon 5	TCTAGAGGGAGA-TGCTGAGTTCAC	0.2 kb
463	13483-L14945	Exon 5	395-396	AGAAAATGGCTT-TCCAGATAGCAC	14.2 kb
202	13484-L15493	Exon 6	615-616	TCCTATGACGAA-TTGTGCTTCCTG	
		stop codon	*805-807 (Exon 6)*		

Table 2d. *WNT10A* gene

Length (nt)	SALSA MLPA probe	*WNT10A* exon[a]	Ligation site NM_025216.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*145-147 (Exon 1)*		
136	16457-L18910	Exon 1	228-229	CTCCTGTTCTTC-CTACTGCTGCTG	1.3 kb
382	16458-L18911	Exon 2	490-491	CTCGCAACAAGA-TCCCCTATGAGA	7.8 kb
474	16459-L18912	Exon 3	692-693	GGATGCACTGCA-GCGTGGTAAGGG	2.6 kb
297	16460-L25670	Exon 4	908-909	GCAGGCAGTGAT-GGAGAACATGCG	
		stop codon	*1396-1398 (Exon 4)*		

[a] See section Exon numbering on page 2 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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ 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.

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.
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Selected publications using SALSA MLPA Probemix P183 EDA-EDAR-EDARADD

- Duan H et al. (2017). Gene screening facilitates diagnosis of complicated symptoms: A case report. *Mol Med Rep*. 16:7915-7922.
- Lexner MO et al. (2008). X-linked hypohidrotic ectodermal dysplasia. Genetic and dental findings in 67 Danish patients from 19 families. *Clin Gen*. 74:252-259.
- Martínez-Romero MC et al. (2019). EDA, EDAR, EDARADD and WNT10A allelic variants in patients with ectodermal derivative impairment in the Spanish population. *Orphanet J Rare Dis*. 14:1-10.

- Monroy-Jaramillo N et al. (2017). Mutational spectrum of EDA and EDAR genes in a cohort of Mexican mestizo patients with hypohidrotic ectodermal dysplasia. *J Eur Acad Dermatol Venereol*. 31:e321-4.
- Wohlfart S et al. (2016). Mutational spectrum in 101 patients with hypohidrotic ectodermal dysplasia and breakpoint mapping in independent cases of rare genomic rearrangements. *J of Hum Gen*. 61:891-897.
- Wohlfart S and Schneider H. (2019). Variants of the ectodysplasin A1 receptor gene underlying homozygous cases of autosomal recessive hypohidrotic ectodermal dysplasia. *Clin Genet*. 95:427-432.

P183 product history	
Version	*Modification*
C1	Probes for the *WNT10A* gene have been included, one *EDAR* probe and one reference probe have been removed, seven reference probes have been replaced and the control fragments have been adjusted (QDX2).
B1	Probes for the *EDAR* and *EDARADD* genes have been included. One *EDA* probe has been replaced and one *EDA* probe has been added. In addition, four extra control fragments have been included.
A1	First release.

Implemented changes in the product description

Version C1-01 – 29 April 2021(04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *EDA, EDAR, EDARADD* and *WNT10A* genes updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- New references added.

Version 11 – 05 July 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new pictures included).
- References added on page 2.
- Exon numbering of the EDA and EDARADD genes has been changed.
- Various minor textual changes.

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