Instructions for Use

SALSA® MLPA® Probemix P137 SCN1A

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P137 SCN1A product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P137 SCN1A**
Version	C1
Catalogue numbers	P137-025R (25 reactions) P137-050R (50 reactions) P137-100R (100 reactions)
Basic UDI-DI	n.a.
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE
RUO	ALL OTHER COUNTRIES

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

More Information: www.mrcholland.com	
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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version B3, probes targeting *SCN1A* exon 3 and 15, and two reference probes have been replaced in version C1.

1. Intended Purpose

The SALSA MLPA Probemix P137 SCN1A is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the human *SCN1A* gene in genomic DNA isolated from human peripheral whole blood specimens. P137 SCN1A is intended to confirm a potential cause for and clinical diagnosis of Dravet syndrome (DS) and other *SCN1A*-related seizure disorders, and Familial hemiplegic migraine 3 (FHM3).

Copy number variations (CNVs) detected with P137 SCN1A should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the SCN1A gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of DS or other *SCN1A*-related disorders, and FHM3.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*SCN1A* gene)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

Approximately 2-3% of all mutations in DS are expected to be deletions/duplication (Marini et al. 2009), which can be detected with the P137-C1 probemix. In *SCN1A*-point mutation negative patients this percentage is approximately 16% (Madia et al. 2006, Wang et al. 2008) (https://www.ncbi.nlm.nih.gov/books/NBK1318/). The percentage of deletions/duplications for other *SCN1A*-related seizures disorders and FHM3 is unknown. However, the association between these diseases and the *SCN1A* gene is well established. Analytical performance for the detection of deletions and duplications in the *SCN1A* gene is very high and can be considered >99% (based on a 2006-2024 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
2q24.3	*SCN1A*	Exon 29 (26)	2.5 kb	445	04547-L18519	
2q24.3	*SCN1A*	Exon 28 (25)	1.8 kb	364	17380-L04745	
2q24.3	*SCN1A*	Exon 27 (24)	2.1 kb	319	04545-L18535	
2q24.3	*SCN1A*	Exon 26 (23)	1.6 kb	280	04544-L04900	
2q24.3	*SCN1A*	Exon 25 (22)	2.9 kb	243	04543-L18518	
2q24.3	*SCN1A*	Exon 24 (21)	7.2 kb	202	04542-L03931	
2q24.3	*SCN1A*	Exon 23 (20)	2.4 kb	172	04541-L03930	
2q24.3	*SCN1A*	Exon 22 (19)	1.7 kb	149	04540-L03929	#
2q24.3	*SCN1A*	Exon 21 (18)	1.9 kb	427	04539-L03928	
2q24.3	*SCN1A*	Exon 20 (17)	20.5 kb	400	04538-L03927	
2q24.3	*SCN1A*	Exon 19 (16)	1.9 kb	373	04537-L03926	
2q24.3	*SCN1A*	Exon 18 (15)	1.4 kb	328	22542-L31769	
2q24.3	*SCN1A*	Exon 17 (14)	1.9 kb	292	04535-L03924	
2q24.3	*SCN1A*	Exon 16 (13)	1.0 kb	250	04534-L30856	
2q24.3	*SCN1A*	Exon 15 (12)	1.5 kb	211	04533-L03922	
2q24.3	*SCN1A*	Exon 14 (11)	1.4 kb	178	04532-L03921	
2q24.3	*SCN1A*	Exon 13 (10)	1.5 kb	154	04531-L05030	
2q24.3	*SCN1A*	Exon 12 (9)	1.0 kb	337	07367-L07014	
2q24.3	*SCN1A*	Exon 11 (8)	1.2 kb	391	04530-L03919	
2q24.3	*SCN1A*	Exon 10 (7)	3.0 kb	355	04529-L03918	
2q24.3	*SCN1A*	Exon 9 (6)	1.0 kb	310	04528-L03917	#
2q24.3	*SCN1A*	Exon 8 (5)	1.7 kb	274	04527-L04899	
2q24.3	*SCN1A*	Exon 7 (4)	1.8 kb	229	04526-L03915	
2q24.3	*SCN1A*	Exon 6 (3)	2.2 kb	196	22541-L31721	#
2q24.3	*SCN1A*	Exon 5 (2)	14.9 kb	166	04524-L03913	
2q24.3	*SCN1A*	Exon 4 (1)	54.3 kb	160	04523-L18517	
2q24.3	*SCN1A*	Exon 1 (hB)	0.2 kb	265	15942-L18068	Ø
2q24.3	*SCN1A*	Exon 1 (hB)	21.1 kb	409	15943-L18069	Ø
2q24.3	*SCN1A*	Upstream (hA)	0.1 kb	183	15940-L18066	Ø
2q24.3	*SCN1A*	Upstream (hA)		236	15941-L18067	Ø
1q	Reference			436	14775-L16472	
3p	Reference			221	07223-L21127	
4q	Reference			256	10808-L27953	
6q	Reference			454	14954-L16687	
7q	Reference			142	09258-L11422	
8q	Reference			190	06743-L06347	
11q	Reference			301	08313-L08182	
12q	Reference			382	04278-L23577	
14q	Reference			418	11057-L11726	
20p	Reference			346	05982-L05407	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *SCN1A* exon numbers are derived from the MANE project and are based on the MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

Ø These probes target sequences outside of the known coding region. Copy number alterations of only these probes are of unknown clinical significance. The significance of deletions/duplications of these probes (previously referred to as exons hA and hB) is not clear as these exons are non-coding. However, microdeletions in these exons have been found in two DS patients, and a point mutation in exon hA has been described in a patient with partial epilepsy with antecedent febrile seizures (Gao et al. 2017, Nakayama et al. 2010).

\# The specificity of these probes rely on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only these probes can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. The *SCN1A* gene is located in a complicated 2q24 region, since several highly homologous genes are present in this region (*SCN2A*, *SCN3A*, *SCN7A* and *SCN9A*). In rare cases, apparent duplications might therefore be due to sequence changes in the other similar genes.
6. Mosaicism has been reported in individuals with DS (de Lange et al. 2018, Nakayama et al. 2018)(Mosaic *SCN1A* cases obtained with the P137 SCN1A probemix must be confirmed by analysis of a second, independently collected DNA sample or a different technique, in order to exclude a false positive mosaic result.

Technique-specific precautions

See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The clinical significance of the following findings is not yet clear/clearly established: deletions or duplications in exon 1 and/or the upstream probe (Gao et al. 2017, Nakayama et al. 2010).
2. The significance of exons 1 to 3 deletions is not clear as these exons are non-coding and alternative transcript variants using other transcription start sites are known.
3. Probes for non-coding exons 2 and 3 are not included in this mix, therefore their association with *SCN1A*-related disorders cannot be determined with this probemix.

Technique-specific limitations

See the MLPA General Protocol.

9. References Cited in this IFU

1. de Lange IM et al. (2018). Mosaicism of de novo pathogenic SCN1A variants in epilepsy is a frequent phenomenon that correlates with variable phenotypes. *Epilepsia.* 59:690-703.
2. Gao QW et al. (2017). A Point Mutation in SCN1A 5' Genomic Region Decreases the Promoter Activity and Is Associated with Mild Epilepsy and Seizure Aggravation Induced by Antiepileptic Drug. *Mol Neurobiol.* 54:2428-2434.
3. Madia F et al. (2006). Cryptic chromosome deletions involving SCN1A in severe myoclonic epilepsy of infancy. *Neurology.* 67:1230-1235.
4. Marini C et al. (2009). SCN1A duplications and deletions detected in Dravet syndrome: implications for molecular diagnosis. *Epilepsia.* 50:1670-1678.
5. Nakayama T et al. (2010). Deletions of SCN1A 5' genomic region with promoter activity in Dravet syndrome. *Hum Mutat.* 31:820-829.
6. Nakayama T et al. (2018). Somatic mosaic deletions involving SCN1A cause Dravet syndrome. *Am J Med Genet A.* 176:657-662.
7. Wang JW et al. (2008). Microchromosomal deletions involving SCN1A and adjacent genes in severe myoclonic epilepsy in infancy. *Epilepsia.* 49:1528-1534.

Implemented changes in the product description

Version C1-03 – 21 February 2025 (03S)

- Product description was adapted to a new template.
- Exon numbering updated for *SCN1A* to MANE exon numbering.
- SNV rs149579028 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes, included in the section 7. Precautions and Warnings