## 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)<br>P137-050R (50 reactions)<br>P137-100R (100 reactions) |
| **Basic UDI-DI** | 872021148P1375Y |
| **Ingredients** | Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA |

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

**Storage and Shelf Life**

| | | |
|---|---|---|
| Recommended conditions | -25°C -15°C | ☼ |

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.

| **Regulatory Status** | |
|---|---|
| IVD | EUROPE CE 2797 |
| RUO | ALL OTHER COUNTRIES |

| **Label Symbols** | | | |
|---|---|---|---|
| IVD | In Vitro Diagnostic | RUO | Research Use Only |

| **More Information:** **www.mrcholland.com** | |
|---|---|
| 🏭 | MRC Holland BV; Willem Schoutenstraat 1<br>1057 DL, Amsterdam, the Netherlands |
| E-mail | info@mrcholland.com (information & technical questions);<br>order@mrcholland.com (orders) |
| Phone | +31 888 657 200 |

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 6 and 18, 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 for molecular genetic testing of at-risk family members. In addition, results of this assay can aid in patient management decisions.

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, parental evaluation, 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.

## 2. Sample Requirements

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

| Sample Types | | |
|---|---|---|
| Test Sample | • Provided by user | |
| Reference Samples (Required) | • Provided by user<br>• Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples.<br>• Have a normal copy number and ≤0.10 standard deviation for all probes.<br>• 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 seizure disorders. | |
| No-DNA Control (Preferably) | • Provided by user<br>• $TE_{0.1}$ buffer instead of DNA<br>• 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.

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[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.

## 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<br>or<br>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

| Study | Description |
|---|---|
| Expected values for copy number in normal and affected populations | To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the product description can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P137 SCN1A in 45 samples from healthy individuals with normal copy number and 8 samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested. |
| Limit of Detection | A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using SALSA MLPA Probemix P137 SCN1A on two samples with known CNVs and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA. |
| Interfering substances | SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.<br>A study using SALSA MLPA Probemix P137 SCN1A was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs and one wild-type sample. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below. |

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td></td><td>
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 270/270 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 250/270 measurements</td></tr>
<tr><td>Fe3+ (FeCl3)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 270/270 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 270/270 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 270/270 measurements</td></tr>
</table>
<p>* Results are summarised for all 30 SCN1A probes across three samples tested in triplicate.</p>
<p>NaCl had the largest effect on copy number determination, as FRs within an ambiguous or incorrect range were found in all samples. DNA extraction methods from blood remove NaCl and during testing of 45 samples extracted from blood, the expected final ratios were found. Therefore, it is only when NaCl is present in excess that deviating results for these probes can be found. Importantly, Coffalyser.Net issued warnings for the samples in which NaCl showed an effect, as well as lowered quality scores.</p>
<p>To minimise variability across samples, 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.</p>
</td></tr>
<tr><td>Cross-reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, five previously genotyped samples were tested using SALSA MLPA Probemix P137 SCN1A and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number status over multiple days, and by multiple operators. Results showed a correct call in 1288/1290 data points, leading to a precision of >99%.</td></tr>
<tr><td>Clinical validity*</td><td><p>Large deletions and duplications in the <i>SCN1A</i> gene explain 2 – 3% of the DS cases, and 0.3 – 2% of cases with other <i>SCN1A</i>-related seizure disorders.</p><p>*(Based on a 2006-2025 literature review)</p></td></tr>
</table>

**Summary of Safety and Performance (SSP)**
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

**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 previous exon numbering using RefSeq transcript NM_06920.4 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, heterozygous microdeletions in this region have been found in two DS patients, and a point mutation in the upstream region 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 relies 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 also present (*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 by a different technique, in order to exclude a false positive mosaic result.
7. Large deletions are often detected with this probemix. The slope correction algorithm in Coffalyser.Net may confuse a large *SCN1A* deletion for sloping, which may lead to an incorrectly applied slope correction and ambiguous results for multiple probes. Please contact MRC Holland if you suspect that the slope correction has been incorrectly applied: info@mrcholland.com.

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 region (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 seizure 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 C1-04 – 13 January 2026 (03S)*<br>- Intended purpose was updated. It was specified that the assay is manual, the intended population was extended to include molecular genetic testing of at-risk family members, and it was specified that the assay can aid in patient management decisions. In addition, parental evaluation was added. Familial hemiplegic migraine type 3 (FHM3) was removed from the intended purpose due to lack of clinical evidence.<br>- Exon numbering in “Changes in this Product Version” adjusted to adhere to MANE Select transcript exon numbering.<br>- Section 6. Performance Characteristics was updated with the addition of a summary table presenting performance data.<br>- Previous exon numbering was based on RefSeq transcript NM_06920.4. This is now mentioned in the legend below the content table.<br>- Precaution 7 was added to specify follow-up steps when signal sloping is present.<br>- “Upstream probe” was changed to “upstream region” in probemix-specific limitation 1.<br>- Probemix is now IVDR certified. |