MRC Holland SALSA® MLPA®

Instructions for Use

SALSA® MLPA® Probemix P138 SLC2A1-STXBP1

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 P138 SLC2A1-STXBP1 product page on our website to find Certificates of Analysis and a list of related products.

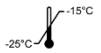
Product Name	**SALSA® MLPA® Probemix P138 SLC2A1-STXBP1**
Version	D1
Catalogue numbers	P138-025R (25 reactions) P138-050R (50 reactions) P138-100R (100 reactions)
Basic UDI-DI	872021148P13862
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
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	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, the Netherlands
E-mail	info@mrcholland.com (information & technical questions); 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 C1, four reference probes have been replaced and one removed. One target probe has been added for *STXBP1* and four probe lengths have been adjusted.

1. Intended Purpose

The SALSA MLPA Probemix P138 SLC2A1-STXBP1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for (1) the detection of deletions in the *SLC2A1* gene in order to confirm a potential cause for and clinical diagnosis of Glucose transporter type 1 deficiency syndrome (GLUT1 DS), and (2) the detection of deletions or duplications in the *STXBP1* gene in order to confirm a potential cause for and clinical diagnosis of STXBP1 Encephalopathy with epilepsy (STXBP1-E) including Ohtahara syndrome (OS). This assay is for use with genomic DNA isolated from human peripheral whole blood specimens.

Copy number variations (CNVs) detected with P138 SLC2A1-STXBP1 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 *SLC2A1* and *STXBP1* genes 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, 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 GLUT1 DS, STXBP1-E and OS.
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

*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 (*SLC2A1*, *STXBP1*)

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

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy number in normal and affected populations</td><td>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 P138 SLC2A1-STXBP1 in 44 samples from healthy individuals with normal copy number and five control plasmid samples with known CNVs. One deviation was observed in a sample from a healthy individual. The expected FRs for the corresponding copy number were found in all but one of the samples tested (98% of samples).</td></tr>
<tr><td>Limit of Detection</td><td>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 P138 SLC2A1-STXBP1 on one sample with no aberrations, and expected results were obtained in 91% of probe measurements using the lower input amount of DNA, and 100% of probe measurements using upper input amount of DNA.</td></tr>
<tr><td>Interfering substances</td><td>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.

A study using P138 SLC2A1-STXBP1 was performed to assess the potential for interference of endogenous and exogenous substances on DNA from control plasmid samples with known CNVs. 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>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>Expected FR for 461/476 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Expected FR for 507/510 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Expected FR for 497/510 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Expected FR for 507/510 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Expected FR for 101/476 measurements</td></tr>
</table>
* Results are summarised for all probes across all five samples tested. All samples were tested in triplicate except for one sample tested in duplicate for EDTA and hemoglobin.

An effect on the FRs was observed for a low number of probes with EDTA, NaCl, Fe^{3+} and heparin. Hemoglobin had the largest effect on the FRs. Coffalyser.Net issued warnings for the samples in which the interferents showed an effect, as well as lowered quality scores, this led to samples needing a re-test.

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.</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, 44 previously genotyped samples were tested using P138 SLC2A1-STXBP1 and found to have the expected results in 99.6% of probe measurements. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 2503/2516 data points, leading to a precision of >99%.</td></tr>
<tr><td>Clinical validity*</td><td>SLC2A1: ~10% GLUT1 DS is caused by deletions in SLC2A1 (Gene Reviews).

STXBP1: >5% of STXBP1-E is caused by deletions and duplications in STXBP1 (Gene Reviews).

*(Based on a 2007-2025 literature review)</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
1p34.2	*SLC2A1*	Exon 10	0.6 kb	319	20672-L28411	
1p34.2	*SLC2A1*	Exon 9	1.2 kb	275	04489-L03878	
1p34.2	*SLC2A1*	Exon 8	0.3 kb	247	04488-L19893	
1p34.2	*SLC2A1*	Exon 7	0.5 kb	202	04487-L03876	
1p34.2	*SLC2A1*	Exon 6	0.3 kb	172	04486-L03875	
1p34.2	*SLC2A1*	Exon 5	0.8 kb	149	23077-L32572	¥
1p34.2	*SLC2A1*	Exon 4	0.3 kb	301	04484-L03873	
1p34.2	*SLC2A1*	Exon 3	12.2 kb	266	04483-L03872	
1p34.2	*SLC2A1*	Exon 2	0.1 kb	238	04482-L19892	
1p34.2	*SLC2A1*	Exon 2	15.3 kb	178	05077-L32570	¥
1p34.2	*SLC2A1*	Exon 1	0.2 kb	232	23145-L32569	¥
1p34.2	*SLC2A1*	Exon 1		214	05075-L32571	¥ Ø
9q34.11	*STXBP1*	Exon 1	0.1 kb	295	23144-L26526	Ø
9q34.11	*STXBP1*	Exon 1	39.3 kb	142	19742-L27154	Ø
9q34.11	*STXBP1*	Exon 2	2.1 kb	436	19744-SP0862-L26527	Ж
9q34.11	*STXBP1*	Exon 3	4.7 kb	373	19745-L26528	
9q34.11	*STXBP1*	Exon 4	1.7 kb	337	19746-L26529	
9q34.11	*STXBP1*	Exon 5	1.0 kb	465	19747-L26530	
9q34.11	*STXBP1*	Exon 6	2.1 kb	427	19748-L26531	
9q34.11	*STXBP1*	Exon 7	2.1 kb	381	19749-L26532	
9q34.11	*STXBP1*	Exon 8	0.9 kb	346	19750-L26533	
9q34.11	*STXBP1*	Exon 9	1.9 kb	418	19751-L26534	
9q34.11	*STXBP1*	Exon 10	1.8 kb	393	19752-L26535	
9q34.11	*STXBP1*	Exon 11	2.2 kb	454	19753-SP0863-L26536	Ж
9q34.11	*STXBP1*	Exon 12	1.1 kb	196	19754-L26537	
9q34.11	*STXBP1*	Exon 13	2.7 kb	154	19755-L26538	
9q34.11	*STXBP1*	Exon 14	0.7 kb	328	19756-L26539	
9q34.11	*STXBP1*	Exon 15	1.8 kb	187	19757-L27151	
9q34.11	*STXBP1*	Exon 16	1.8 kb	226	19758-L26541	
9q34.11	*STXBP1*	Exon 17	2.3 kb	283	19759-L26542	
9q34.11	*STXBP1*	Exon 18	2.0 kb	364	19760-L26543	
9q34.11	*STXBP1*	Intron 18	6.7 kb	411	19761-L26544	Ø
9q34.11	*STXBP1*	Exon 19	0.1 kb	160	19762-L26545	Ø
9q34.11	*STXBP1*	Exon 19		208	19763-L26546	Ø
1p	Reference			445	15733-L17713	
3q	Reference			130	21397-L29874	
5q	Reference			166	07904-L27150	
7q	Reference			257	04594-L03773	
10q	Reference			220	21057-L30157	
12p	Reference			355	11614-L12374	
15q	Reference			402	11021-L11690	
16p	Reference			310	21216-L29591	
17q	Reference			472	11200-L15331	

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 *SLC2A1*/*STXBP1* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with targets at the edge of or slightly outside the coding region were altered.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe changes

¥ Probe(s) changed in this product version. Minor alteration, no change in sequence detected.

Probe warnings

Ж These probes consist of three parts and have two ligation sites. A low signal of these probes 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.

Ø This probe targets a sequence outside of the known coding region.

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. *De novo* pathogenic variants in *STXBP1* or *SLC2A1* may also be the result of germline mosaicism in apparently unaffected parents (Saitsu et al. 2011; Takahashi et al. 2017). Mosaic cases 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 and limitations
See the MLPA General Protocol.

8. References Cited in this IFU

1. Saitsu H et al. (2011). Paternal mosaicism of an STXBP1 mutation in OS. *Clin Genet.* 80:484-488.
2. Takahashi S et al. (2017). Somatic mosaicism for a SLC2A1 mutation: implications for genetic counseling for GLUT1 deficiency syndrome. *Clin Genet.* 91:932-933

Implemented changes in the product description
Version D1-03 – 01 April 2026 (03S) - Removed detection of duplications in the *SLC2A1* gene from the intended purpose and updated with minor textual changes. - Performance Characteristics section updated with data from analytical performance experiments. - Warning added for probes 05075-L32571, 23144-L26526 and 19742-L27154, targeting regions with outside of the known coding region. - Warnings for new probes removed. - Exon numbering from version D1-02 in brackets removed. - Rephrased the Probemix-specific precaution on parental mosaicism. - Updated references cited in this IFU. - Minor textual changes. - Probemix is now IVDR certified.