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.

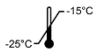
Product Name	**SALSA® MLPA® Probemix P138 SLC2A1-STXBP1**
Version	D1
Catalogue numbers	P138-025R (25 reactions) P138-050R (50 reactions) P138-100R (100 reactions)
Basic UDI-DI	n.a.
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 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	
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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 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 assay[2] for (1) the detection of deletions or duplications 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 human *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, 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 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

Approximately 13% of all mutations in GLUT1 DS are expected to be deletions in *SLC2A1* gene (https://www.ncbi.nlm.nih.gov/books/NBK1430/), which can be detected with the P138 probemix. The percentage of deletions/duplications for STXBP1-E and OS is unknown. However, the association between STXBP1-E and OS and the *STXBP1* gene is well established (Beal et al. 2012, Saitsu et al. 2008). Analytical performance for the detection of deletions/duplications in the *SLC2A1* and *STXBP1* genes 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
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	¥
9q34.11	*STXBP1*	Exon 1	0.1 kb	295	23144-L26526	*
9q34.11	*STXBP1*	Exon 1	39.3 kb	142	19742-L27154	
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 (Exon 19)	6.7 kb	411	19761-L26544	Ø
9q34.11	*STXBP1*	Exon 19 (Exon 20)	0.1 kb	160	19762-L26545	Ø
9q34.11	*STXBP1*	Exon 19 (Exon 20)		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. Annotation of one probe with a target at the edge of or slightly outside the coding region, is altered. 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 changes

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

Probe warnings

Ж	These probes consist of three parts and has 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. Copy number alterations of only this probe are of unknown clinical significance.

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. Mosaicism has been reported in individuals with OS. Mosaic STXBP1 cases obtained with the P138 SLC2A1-STXBP1 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.

Technique-specific limitations
See the MLPA General Protocol.

8. References Cited in this IFU

1. Beal JC et al. (2012). Early-onset epileptic encephalopathies: Ohtahara syndrome and early myoclonic encephalopathy. Pediatr Neurol. 47:317-323.
2. Saitsu H et al. (2008). De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat Genet. 40:782-788.

Implemented changes in the product description
Version D1-02 – 21 February 2025 (03S) - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Exon numbering updated to MANE Select for *STXBP1* gene probes. - Warning added for probe 19761-L26544 with copy number alterations of unknown clinical significance. - Warnings for salt sensitive probes removed as no longer applicable (23144-L26526 and 19742-L27154).