Product Description
SALSA® MLPA® Probemix P138-D1 SLC2A1-STXBP1

To be used with the MLPA General Protocol.

Version D1
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. For complete product history see page 10.

Catalogue numbers:
- **P138-025R:** SALSA MLPA Probemix P138 SLC2A1-STXBP1, 25 reactions.
- **P138-050R:** SALSA MLPA Probemix P138 SLC2A1-STXBP1, 50 reactions.
- **P138-100R:** SALSA MLPA Probemix P138 SLC2A1-STXBP1, 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.

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 in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Glucose transporter type 1 deficiency syndrome (GLUT1 DS; OMIM #606777) is a neurologic disorder with broad phenotypic variability. GLUT1 DS is usually inherited in an autosomal dominant manner, and in very rare cases, it can occur as an autosomal recessive disease. About 10% of the autosomal dominant cases have a clinically affected parent, the remaining 90% results from a *de novo* heterozygous pathogenic variant. The *de novo* sporadic mutations may also be the result of germline mosaicism in apparently unaffected parents (Takahashi et al. 2017).

The GLUT1 DS phenotype has been reported as classic (~90%) and non-classic (~10%). The classic phenotype is characterised by infantile-onset seizures (usually between 1-6 months), delayed neurologic development, dysarthria, acquired microcephaly, and movement disorders (ataxia, dystonia, chorea). The non-classic phenotype is milder and is characterised by absence of clinical seizures and frequent paroxysmal dyskinesias (intermittent ataxia, choreoathetosis, dystonia, alternating hemiplegia). More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1430/.

STXBP1 Encephalopathy with epilepsy (*STXBP1*-E), also known as *STXBP1* Encephalopathy or Early infantile epileptic encephalopathy-4 (EIEE4; OMIM #612164), belongs to the genetically heterogeneous group of early infantile epileptic encephalopathy (EIEE) disorders. *STXBP1*-E is inherited in an autosomal dominant pattern, with most of the cases being the result of a *de novo* mutation in the *STXBP1* gene and with no history of the disorder in their family. However, these *de novo* sporadic mutations may also be the result of germline mosaicism in apparently unaffected parents (Saitsu et al. 2011).

STXBP1-E is characterised by recurrent seizures with early-onset (from day 1 to 13 years of age in 94% of the cases), global development delay (90% of the cases), abnormal brain function and intellectual disability. Electroencephalography (EEG) abnormalities such as burst suppression pattern and hypsarrhythmia were reported in affected individuals. The most common type of seizures is infantile spasms that consist of involuntary muscles spasms. Several epileptic syndromes have been linked to the *STXBP1* gene, such as Ohtahara syndrome (OS; the most common syndrome, with *STXBP1* defects being reported in ~20% of OS patients), West syndrome (WS), Lennox-Gastaut syndrome (LGS), Dravet syndrome (not *SCN1A*-related), classic Rett syndrome (not *MECP2*-related) and atypical Rett syndrome (not *CDKL5*-related). OS can develop into WS, this transition is accompanied by changes in the EEG, from suppression burst (OS) to hypsarrhythmia (WS). When progression continues to LGS, a generalized slow spike-wave pattern develops. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK396561/.

Gene structure
The *SLC2A1* spans ~34 kilobases (kb) on chromosome 1p34.2 and contains 10 exons. The *SLC2A1* LRG_1132 is available at www.lrg-sequence.org and is identical to GenBank NG_008232.1.

The *STXBP1* spans ~80 kb on chromosome 9q34.11 and contains 20 exons. No LRG is available at www.lrg-sequence.org. *STXBP1* GenBank NG_016623.1 is available.

Transcript variants
For *SLC2A1,* one transcript variant has been described encoding the full length protein (NM_006516.4; 3384 nt; coding sequence 218-1696; https://www.ncbi.nlm.nih.gov/gene/6513). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 10.

For *STXBP1,* multiple variants have been described. Transcript variant 1a is the most predominant and encodes isoform *1a* (NM_003165.6; 3880 nt; coding sequence 128-1939; https://www.ncbi.nlm.nih.gov/gene/6812). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 19.

Exon numbering
The *SLC2A1* exon numbering used in this P138-D1 SLC2A1-STXBP1 product description is the exon numbering from the LRG_1132 sequence. The *STXBP1* exon numbering used is the exon numbering from the RefSeq transcript NM_003165.6. 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 LRG 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 P138-D1 SLC2A1-STXBP1 contains 43 MLPA probes with amplification products between 130 and 472 nucleotides (nt). This includes 12 probes for the *SLC2A1* gene and 22 probes for the *STXBP1* gene covering each exon of these genes. 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 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 from human peripheral whole blood, 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 GLUT1 DS, *STXBP1*-E and OS. 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. The quality of cell lines can change; therefore samples should be validated before use.

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 Ohtahara syndrome 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 2006-2020 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.

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 expected results for the *SLC2A1* and *STXBP1* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). Copy numbers of 4 (heterozygous triplication/homozygous duplication) may occur, but are extremely rare. A copy number of 0 (homozygous deletion) of these genes is unlikely to be found in blood derived DNA, as such a deletion is expected to be embryonically lethal.

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 for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous 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.

P138 specific note:
- 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.

Limitations of the procedure
- In most populations, the major cause of genetic defects in the *SLC2A1* and *STXBP1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P138 SLC2A1-STXBP1.
- 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.

LOVD mutation database
https://databases.lovd.nl/shared/genes/SLC2A1 and https://databases.lovd.nl/shared/genes/STXBP1. We strongly encourage users to deposit positive results in the LOVD Database (Leiden Open Variation Database 3.0). 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 *SLC2A1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P138-D1 SLC2A1-STXBP1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*SLC2A1*	*STXBP1*
64-105	Control fragments – see table in probemix content section for more information			
130 *	Reference probe 21397-L29874	3q		
142 «	**STXBP1 probe** 19742-L27154			**Exon 1**
149 ¥	**SLC2A1 probe** 23077-L32572		**Exon 5**	
154	**STXBP1 probe** 19755-L26538			**Exon 13**
160 ◎	**STXBP1 probe** 19762-L26545			**Exon 20**
166	Reference probe 07904-L27150	5q		
172	**SLC2A1 probe** 04486-L03875		**Exon 6**	
178 ¥	**SLC2A1 probe** 05077-L32570		**Exon 2**	
187	**STXBP1 probe** 19757-L27151			**Exon 15**
196	**STXBP1 probe** 19754-L26537			**Exon 12**
202	**SLC2A1 probe** 04487-L03876		**Exon 7**	
208 ◎	**STXBP1 probe** 19763-L26546			**Exon 20**
214 ¥	**SLC2A1 probe** 05075-L32571		**Exon 1**	
220 *	Reference probe 21057-L30157	10q		
226	**STXBP1 probe** 19758-L26541			**Exon 16**
232 ¥	**SLC2A1 probe** 23145-L32569		**Exon 1**	
238	**SLC2A1 probe** 04482-L19892		**Exon 2**	
247	**SLC2A1 probe** 04488-L19893		**Exon 8**	
257	Reference probe 04594-L03773	7q		
266	**SLC2A1 probe** 04483-L03872		**Exon 3**	
275	**SLC2A1 probe** 04489-L03878		**Exon 9**	
283	**STXBP1 probe** 19759-L26542			**Exon 17**
295 * «	**STXBP1 probe** 23144-L26526			**Exon 1**
301	**SLC2A1 probe** 04484-L03873		**Exon 4**	
310 *	Reference probe 21216-L29591	16p		
319	**SLC2A1 probe** 20672-L28411		**Exon 10**	
328	**STXBP1 probe** 19756-L26539			**Exon 14**
337	**STXBP1 probe** 19746-L26529			**Exon 4**
346	**STXBP1 probe** 19750-L26533			**Exon 8**
355	Reference probe 11614-L12374	12p		
364	**STXBP1 probe** 19760-L26543			**Exon 18**
373	**STXBP1 probe** 19745-L26528			**Exon 3**
381	**STXBP1 probe** 19749-L26532			**Exon 7**
393	**STXBP1 probe** 19752-L26535			**Exon 10**
402	Reference probe 11021-L11690	15q		
411	**STXBP1 probe** 19761-L26544			**Exon 19**
418	**STXBP1 probe** 19751-L26534			**Exon 9**
427	**STXBP1 probe** 19748-L26531			**Exon 6**
436 Ж	**STXBP1 probe** 19744-SP0862-L26527			**Exon 2**
445 *	Reference probe 15733-L17713	1p		
454 Ж	**STXBP1 probe** 19753-SP0863-L26536			**Exon 11**
465	**STXBP1 probe** 19747-L26530			**Exon 5**
472	Reference probe 11200-L15331	17q		

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

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
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.
◎ The clinical significance of *STXBP1* exon 20 deletions/duplications is not clear as this exon is non-coding.

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. P138-D1 probes arranged according to chromosomal location

Table 2a. *SLC2A1*

Length (nt)	SALSA MLPA probe	*SLC2A1* exon[a]	Ligation site NM_006516.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*218-220 (Exon 1)*		
214	05075-L32571	Exon 1	78-79	AGGGAGCAGGAG-ACCAAACGACGG	0.2 kb
232	23145-L32569	Exon 1	2 nt after exon 1	CAGCAGCAAGGT-GAGTCGCGCGCC	15.3 kb
178	05077-L32570	Exon 2	10 nt before exon 2	AGTGTGGTTTGT-TTCTCCGCAGAA	0.1 kb
238	04482-L19892	Exon 2	283-284	GCAGTGCTTGGC-TCCCTGCAGTTT	12.2 kb
266	04483-L03872	Exon 3	436-437	GCCATCTTTTCT-GTTGGGGGCATG	0.3 kb
301	04484-L03873	Exon 4	573-574	GGGCAAGTCCTT-TGAGATGCTGAT	0.8 kb
149	23077-L32572	Exon 5	795-796	GCTGAGCATCAT-CTTCATCCCGGC	0.3 kb
172	04486-L03875	Exon 6	969-970	GAGTCGGCAGAT-GATGCGGGAGAA	0.5 kb
202	04487-L03876	Exon 7	1141-1142	CAGCCTGTGTAT-GCCACCATTGGC	0.3 kb
247	04488-L19893	Exon 8	1243-1244	ATAGGCCTCGCT-GGCATGGCGGGT	1.2 kb
275	04489-L03878	Exon 9	1323-1324	CTATCTGAGCAT-CGTGGCCATCTT	0.6 kb
319	20672-L28411	Exon 10	1533-1534	CATCTTCACTGT-GCTCCTGGTTCT	
		stop codon	*1694-1696 (Exon 10)*		

Table 2b. *STXBP1*

Length (nt)	SALSA MLPA probe	*STXBP1* exon[a]	Ligation site NM_003165.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*128-130 (Exon 1)*		
295 «	23144-L26526	Exon 1	14-15	TCGGTCCCTAGC-GCGGCTGCGGGG	0.1 kb
142 «	19742-L27154	Exon 1	100-101	GGGACGAGGAGA-TCGGAGCCGGGA	39.3 kb
436 Ж	19744-SP0862-L26527	Exon 2	202-203; 18 nt after exon 2	GTCAAGAAGAAG-30 nt spanning oligo-TAATGGGCTTAA	2.1 kb
373	19745-L26528	Exon 3	222-223	GCAGGTGCTGGT-GGTGGATCAGTT	4.7 kb
337	19746-L26529	Exon 4	348-349	CCTGGAGGCTGT-GTATCTCATCAC	1.7 kb
465	19747-L26530	Exon 5	441-442	TGCACACGTCTT-CTTCACTGACTG	1.0 kb
427	19748-L26531	Exon 6	495-496	AAAATCCCGAGC-AGCCAAAGTCAT	2.1 kb
381	19749-L26532	Exon 7	598-599	CAAAGCTTCTAC-AGTCCCCACAAG	2.1 kb
346	19750-L26533	Exon 8	764-765	TCGATGCCTATA-AAGCTGATGATC	0.9 kb
418	19751-L26534	Exon 9	817-818	CGCTCCCAGCTC-CTGATCCTGGAT	1.9 kb
393	19752-L26535	Exon 10	949-950	ATCGGGGAGGCA-CGGGTGAAGGAG	1.8 kb
454 Ж	19753-SP0863-L26536	Exon 11	1058-1059; 4 nt after exon 11	TGAAAGATTTTT-36 nt spanning oligo-ACCCATGCCAGT	2.2 kb
196	19754-L26537	Exon 12	1132-1133	AAGAAGATGCCT-CAGTACCAGAAA	1.1 kb
154	19755-L26538	Exon 13	2 nt before exon 13	GTGCTTTTTCCT-AGTACTCCACCC	2.7 kb
328	19756-L26539	Exon 14	1361-1362	TCCTTCTCTACA-TCTTTTTGAAGA	0.7 kb
187	19757-L27151	Exon 15	1402-1403	AACCTGAACAAA-CTGATCCAGCAC	1.8 kb
226	19758-L26541	Exon 16	1490-1491	GCATGCAGTCCA-CGCTGCGTCGCC	1.8 kb
283	19759-L26542	Exon 17	1629-1630	ACACTACCCTTA-TATCTCTACCCG	2.3 kb
364	19760-L26543	Exon 18	1713-1714	CAAGGCCCCAGG-CGAGTACCGCAG	2.0 kb
411	19761-L26544	Exon 19	1868-1869	AATTTCTCATGG-ACCTGAGACACC	6.7 kb
160 ◎	19762-L26545	Exon 20	2303-2304	GCACAGCAGGAG-AAATTTCTGGAC	0.1 kb
208 ◎	19763-L26546	Exon 20	2384-2385	ACCCTGGGTGAC-GTGCACATTGCT	
		stop codon	*1937-1939 (Exon 19)*		

[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.

¥ Changed in version D1. Minor alteration, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
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.
ⵙ The clinical significance of exon 20 deletions/duplications is not clear as this exon is non-coding.

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.

Related SALSA MLPA probemixes

P015 MECP2	Classic and atypical Rett syndrome, MECP2 duplication syndrome, X-linked intellectual disability syndrome; *MECP2*, *NTNG1*, *CDKL5* and *ARX*.
P137 SCN1A	Dravet syndrome; gene included: *SCN1A*.
P166 KCNQ2	Benign familial neonatal convulsions (BFNC); gene included: *KCNQ2*.
P189 CDKL5/ARX/FOXG1	Atypical Rett syndrome, X-linked infantile spasm syndrome, FOXG1 syndrome; genes included: *CDKL5*, *NTNG1*, *ARX* and *FOXG1*.
P197 KCNQ3	Benign familial neonatal convulsions type 2 (BFNC2); genes included: *KCNQ3, CHRNA4, EPM2A, NHLRC1 (EPM2B)* and *CHRNB2*.
P330 PCDH19	Early Infantile Epileptic Encephalopathy-9 (EIEE9); gene included: *PCDH19*.

References

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- Parrini E et al. (2017). Diagnostic Targeted Resequencing in 349 Patients with Drug-Resistant Pediatric Epilepsies Identifies Causative Mutations in 30 Different Genes. Hum Mutat. 38:216-225.
- Vermeer S et al. (2007). A novel microdeletion in 1(p34.2p34.3), involving the SLC2A1 (GLUT1) gene, and severe delayed development. Dev Med Child Neurol. 49:380-384.

P138 product history	
Version	*Modification*
D1	Four reference probes have been replaced and one removed. One target probe has been added for *STXBP1* and four probe lengths have been adjusted.
C1	Probes for the *STXBP1* gene were added, one reference probe was removed and eight reference probes were replaced. In addition the name of the probemix was changed to indicate the inclusion of the *STXBP1* gene.
B2	Two reference probes were replaced.
B1	Nine reference probes were replaced and four SLC2A1 probes had a small change in length. Extra control fragments (QDX2) were included.
A1	First release.

Implemented changes in the product description
Version D1-01 – 09 July 2021(04P) - Product description adapted to a new product version (version number changed, product history updated, changes in Table 1 and Table 2a and 2b). - UK has been added to the list of countries in Europe that accept the CE mark. Version C1-03 – 10 December 2020 (04P) - Product description adapted to a new template. - Various minor textual changes. - Intended purpose updated. - Ligation sites of the probes targeting the *STXBP1* gene updated according to new version of the NM_ reference sequence. - Information on sub-bands of reference probes was removed from Table 1. - Text used in the warning for more variable probe of probes 295 nt and 178 nt was updated. - Note for mosaicism added to the interpretation of results section. - Performance characteristics section updated. - Text regarding copy number of zero in Interpretation of results section updated. Version C1-02 – 22 April 2020 (04) - Product is now registered for IVD use in Israel. - Version C1-01 – 19 July 2018 (04) - Product description restructured and adapted to a new template. - *SLC2A1* ligation sites adjusted according to the NM_006516.3 RefSeqGene sequence. - Several references removed, 4 references added. - Warning added to Table 1 and Table 2a for more variable probes. - Warning added to Table 2 for the clinical significance of the non-coding exon 20. - Warning removed from Table 1, 445 nt probe 04738-L20091. - Warning for new probes removed from Table 1 and Table 2b. - Version 12 – 20 January 2017 (55) - Warning added to Table 1, 445 nt probe 04738-L20091.

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IVD	EUROPE* CE ISRAEL
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.