Product Description
SALSA® MLPA® Probemix P117-C3 ABCC8

To be used with the MLPA General Protocol.

Version C3
As compared to version C2, one reference probe has been replaced and six probe lengths have been adjusted. For complete product history see page 8.

Catalogue numbers:
- **P117-025R:** SALSA MLPA Probemix P117 ABCC8, 25 reactions.
- **P117-050R:** SALSA MLPA Probemix P117 ABCC8, 50 reactions.
- **P117-100R:** SALSA MLPA Probemix P117 ABCC8, 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.

General information
The SALSA MLPA Probemix P117 ABCC8 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *ABCC8* gene, which is associated with familial hyperinsulinemic hypoglycemia 1.

Familial hyperinsulinemic hypoglycemia 1 (HHF1) is familial hyperinsulinism caused by mutations in the gene encoding the SUR1 subunit of the pancreatic beta cell inwardly rectifying potassium channel (*ABCC8*). Familial hyperinsulinism causes low blood glucose concentrations in infancy due to unregulated insulin release.

Mutations can be divided into two classes. Class I mutations result in the absence of the protein at the surface of the cell membrane and class II mutations cause an always closed channel at the surface of the cell membrane. The latter class gives a less severe phenotype than the former class (Flanagan et al. 2009). ATP-binding cassette, subfamily C, member 8 (ABCC8), together with the pore-forming KCNJ11, forms the pancreatic subtype of K_{ATP} channels. ATP-sensitive potassium channels (K_{ATP}) link membrane potential to cellular metabolism like insulin secretion and neurotransmitter release, by regulating the flux of potassium ions across the cell membrane. They are located in pancreas, heart and vascular smooth muscle tissue.

The *ABCC8* gene (39 exons) spans ~84 kb of genomic DNA and is located on chromosome 11p15.1, ~17 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1375/.

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *ABCC8* exon numbering used in this P117-C3 ABCC8 product description is the exon numbering from the NG_008867.1 sequence. 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 NG 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 P117-C3 ABCC8 contains 49 MLPA probes with amplification products between 131 and 490 nucleotides (nt). This includes 39 probes for the gene *ABCC8*, one for each exon. In addition, 10 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, 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 familial hyperinsulinism. 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.

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 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 or in or near the *ABCC8* gene. 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *ABCC8* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P117 ABCC8.
- 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.

***ABCC8* mutation database**

https://databases.lovd.nl/shared/genes/ABCC8. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 *ABCC8* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P117-C3 ABCC8

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*ABCC8*
64-105	Control fragments – see table in probemix content section for more information		
131 ¥	Reference probe 00797-L25925	5q	
136 ¥	**ABCC8** probe 08103-L32316		**Exon 2**
142 «	**ABCC8** probe 20996-L29385		**Exon 38**
148	**ABCC8** probe 08111-L07987		**Exon 10**
154	**ABCC8** probe 08124-L08000		**Exon 23**
160 «	**ABCC8** probe 08140-L25572		**Exon 39**
166	**ABCC8** probe 09859-L10282		**Exon 18**
172	**ABCC8** probe 08106-L25573		**Exon 5**
178	Reference probe 18905-L24500	1p	
184 «	**ABCC8** probe 08135-L25575		**Exon 34**
190	**ABCC8** probe 08129-L25574		**Exon 28**
195 *	Reference probe 20880-L14405	21q	
202	**ABCC8** probe 19121-L25068		**Exon 21**
208	**ABCC8** probe 20997-L29386		**Exon 1**
214	**ABCC8** probe 08107-L09400		**Exon 6**
220	**ABCC8** probe 08120-L07996		**Exon 19**
226	**ABCC8** probe 19123-L25070		**Exon 13**
232	**ABCC8** probe 19124-L25071		**Exon 9**
238	**ABCC8** probe 08113-L25708		**Exon 12**
244	Reference probe 13389-L14846	6q	
251 «	**ABCC8** probe 19125-L25072		**Exon 33**
260 ¥ «	**ABCC8** probe 21876-L32314		**Exon 36**
268 ¥	**ABCC8** probe 23010-L32452		**Exon 15**
277	**ABCC8** probe 08126-L25936		**Exon 25**
283 «	**ABCC8** probe 08132-L09469		**Exon 31**
292	Reference probe 09054-L09308	4q	
300	Reference probe 10095-L10519	8q	
310	**ABCC8** probe 08104-L25577		**Exon 3**
319 «	**ABCC8** probe 08133-L25578		**Exon 32**
328 Ж	**ABCC8** probe 18302-SP0638-L25966		**Exon 14**
338	**ABCC8** probe 08105-L25971		**Exon 4**
346	**ABCC8** probe 08127-L08003		**Exon 26**
355	**ABCC8** probe 19127-L25074		**Exon 8**
364 ¥ «	**ABCC8** probe 23011-L32521		**Exon 37**
370	**ABCC8** probe 08118-L25580		**Exon 17**
382	**ABCC8** probe 20998-L25937		**Exon 24**
391	**ABCC8** probe 08131-L26135		**Exon 30**
400	Reference probe 10411-L12204	9q	
409	Reference probe 03272-L02709	3q	
418	**ABCC8** probe 19128-L25075		**Exon 22**
427	Reference probe 11381-L12106	17q	
436	**ABCC8** probe 08117-L07993		**Exon 16**
442	**ABCC8** probe 19118-L25583		**Exon 7**
450 ¥ «	**ABCC8** probe 21487-L32315		**Exon 35**
459	**ABCC8** probe 08112-L26172		**Exon 11**
466	**ABCC8** probe 08128-L26170		**Exon 27**
474	**ABCC8** probe 19129-L25076		**Exon 29**
481	**ABCC8** probe 19130-L25584		**Exon 20**
490	Reference probe 10218-L10698	7q	

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

* New in version C3.
¥ Changed in version C3. 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.

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. *ABCC8* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*ABCC8* exon[a]	Ligation site NM_000352.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*70-72 (Exon 1)*		kb
208	20997-L29386	Exon 1	190-191	TCTTCCTACTCT-TCATCACCTTCC	1.7 kb
136 ¥	08103-L32316	Exon 2	328-329	TCGTCCTGGTGT-GTGAGATTGCAG	4.8 kb
310	08104-L25577	Exon 3	456-457	AACATCGAGACT-TCCAACTTCCCC	6.6 kb
338	08105-L25971	Exon 4	604-605	TGGTGATCCTCT-ATGGGATGCTGC	1.8 kb
172	08106-L25573	Exon 5	794-793 *reverse*	CGATGGGCTTCT-TGTGGGCAGTCT	1.2 kb
214	08107-L09400	Exon 6	1049-1050	CGTGGACCACCT-TGGGAAGGAGAA	7.4 kb
442	19118-L25583	Exon 7	1194-1195	TTTCTGCAAGCA-TCCTACTATGTG	4.6 kb
355	19127-L25074	Exon 8	1377-1378	TTCTTGTGCCCA-AACCTCTGGGCT	5.2 kb
232	19124-L25071	Exon 9	21 nt before exon 9	TCAAAGGGACCT-GACCCATGACCC	0.5 kb
148	08111-L07987	Exon 10	1615-1616	ACGCCTGGGAGA-ACATCTTCCGCA	10.6 kb
459	08112-L26172	Exon 11	1701-1702	TCCTCTGCAGTT-TTCATGAACACG	1.4 kb
238	08113-L25708	Exon 12	1848-1849	CTGTTCCTGCTG-TCCAGTGTGGTC	2.2 kb
226	19123-L25070	Exon 13	1894-1893 *reverse*	CTCGCTTAGCTT-TTGCACGCTGCT	0.4 kb
328 Ж	18302-SP0638-L25966	Exon 14	12nt; 39nt after exon 14	GTGAGTCCTGCT-CTCCCAGAGGGA	0.4 kb
268 ¥	23010-L32452	Exon 15	2165-2166	ACTGTCCAACAT-CACCATTCGTAT	0.8 kb
436	08117-L07993	Exon 16	2282-2281 *reverse*	ACCTGCTCCAGA-AGACAGCCCCTG	10.1 kb
370	08118-L25580	Exon 17	2309-2310	TGACAGCGAGAT-AGGAGAGGACCC	1.5 kb
166	09859-L10282	Exon 18	130 nt before exon 18	CTATGCAGCATT-TGTGGCTACAGA	0.9 kb
220	08120-L07996	Exon 19	2428-2429	TGGAGGAGAACA-TCATCTTTGAGA	1.1 kb
481	19130-L25584	Exon 20	7 nt after exon 20	AACGGGTTAGTA-GCAGCCTCTGAG	0.6 kb
202	19121-L25068	Exon 21	19 nt before exon 21 *reverse*	CAAAGAGGAGGA-ACACATCATGCC	2.2 kb
418	19128-L25075	Exon 22	2718-2719	AAGAGGACAGTG-GTCTTAGTGACC	2.1 kb
154	08124-L08000	Exon 23	2839-2840	AATGCCAGCTCT-TTGAGCACTGGA	1.1 kb
382	20998-L25937	Exon 24	2987-2988	GGAAGAGGAGGA-AGGTACAGGCAA	0.2 kb
277	08126-L25936	Exon 25	3015-3016	AGCGAGGAGGAT-GACAACCTGTCG	0.4 kb
346	08127-L08003	Exon 26	3321-3322	ACGTCTGTCACT-GTGGAGTGGACA	1.2 kb
466	08128-L26170	Exon 27	2 nt after exon 27	CATCGACCAGGT-ACAGAGGACCGT	0.8 kb
190	08129-L25574	Exon 28	33 nt before exon 28	TCAATACCAAAA-TTCACCTCACTC	2.1 kb
474	19129-L25076	Exon 29	22 nt after exon 29	ACATTCCCCCAA-GGTAGGAGTGGA	4.3 kb
391	08131-L26135	Exon 30	3779-3780	CAACATTGCTTC-CCTCTTCCTCAC	0.6 kb
283 «	08132-L09469	Exon 31	3827-3826 *reverse*	ATGCACCGATGT-ACTCCTGGGGAG	0.5 kb
319 «	08133-L25578	Exon 32	3977-3978	GAACCTGGCAGA-CATGGAGCTCCA	0.3 kb
251 «	19125-L25072	Exon 33	4115-4116	GATCCAGAACCT-GAGCGTGCGCTA	1.1 kb
184 «	08135-L25575	Exon 34	4235-4236	CTCCTTCTCTCT-TGCCTTCTTCCG	0.3 kb
450 ¥ «	21487-L32315	Exon 35	55 nt after exon 35	TCAGTTCCATCA-GATCTGGAGCAC	0.3 kb
260 ¥ «	21876-L32314	Exon 36	4379-4380	CACTTCCAGATT-TAACCTGGACCC	1.0 kb
364 ¥ «	23011-L32521	Exon 37	4576-4577	CCAGCATCTTCA-TCATGGACGAGG	0.5 kb
142 «	20996-L29385	Exon 38	14 nt before exon 38	CAACAGCTGTTG-CCCCCACTTGGC	0.8 kb
160 «	08140-L25572	Exon 39	4814-4815	TGCAGACAAGTG-ACCTGCCAGAGC	
		stop codon	*4813-4815 (Exon 39)*		

[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 C3. 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.

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References

- Flanagan SE et al. (2009). Update of mutations in the genes encoding the pancreatic beta-cell K(ATP) channel subunits Kir6. 2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. *Hum Mutat*. 30:170-180.
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- Yorifuji T et al. (2013). Efficacy and safety of long-term, continuous subcutaneous octreotide infusion for patients with different subtypes of KATP-channel hyperinsulinism. *Clin Endocrinol (Oxf)*. 78:891-897.

P117 product history	
Version	*Modification*
C3	One reference probe has been replaced and six probe lengths have been adjusted.
C2	Six probes have been adjusted in length.
C1	Four reference probes have been replaced and four added. Ten target specific probes have been replaced. The control fragments have been adjusted (QDX2).
B1	The probe for *ABCC8* exon 18 has been replaced.
A1	First release.

Implemented changes in the product description
Version C3-01 – 19 January 2021 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *ABCC8* gene updated according to new version of the NM_ reference sequence. *Version 06 (55) – 13 February 2017* - Product description adapted to a new product version (version number changed, lot number added, new picture included). - Minor textual changes on page 1 and 2. - Ligation sites updated according to version 4 of NM_000352. - References added on page 1.

More information: www.mrcholland.com; www.mrcholland.eu	
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