Product Description SALSA® MLPA® Probemix P166-C2 KCNQ2

To be used with the MLPA General Protocol.

Version C2. Compared to previous version, six reference probes have been replaced and four probe lengths have been adjusted. For complete product history see page 6.

Catalogue numbers:
- **P166-025R:** SALSA MLPA Probemix P166 KCNQ2, 25 reactions.
- **P166-050R:** SALSA MLPA Probemix P166 KCNQ2, 50 reactions.
- **P166-100R:** SALSA MLPA Probemix P166 KCNQ2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P166 KCNQ2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *KCNQ2* gene, which is associated with Benign familial neonatal convulsion (BFNC).

Benign familial neonatal convulsion (BFNC) is a rare autosomal dominant disorder caused by defects in the *KCNQ2* gene. BFNC is also known as benign neonatal epilepsy-1 (EBN1), and characterized by clusters of seizures occurring in the first days of life. Most patients have spontaneous remission by 12 months of age.

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/

Probemix content: The SALSA MLPA Probemix P166-C2 KCNQ2 contains 32 MLPA probes with amplification products between 130 and 391 nt. This includes 18 probes for the *KCNQ2* gene, one probe for each exon of the gene. Furthermore, it also contains three probes upstream of the *KCNQ2* gene and one probe downstream of the *KCNQ2* gene. In addition, ten reference probes are included and detect ten different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)
88-96	D-fragments (Low signal of 88 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls results in only five major peaks shorter than 105 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 105 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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: 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 unrelated individuals who are from families without a history of benign familial neonatal convulsion. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the first exon of the *KCNQ2* 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 (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 are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *KCNQ2* gene are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemix P166 KCNQ2.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *KCNQ2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P166-C2 KCNQ2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*KCNQ2*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L13645	5q31	
136	Reference probe 01662-L01237	11q23	
142 «	**KCNQ2 probe** 05522-L05458		**Upstream**
148	Reference probe 03267-L02704	3q29	
159 «	**KCNQ2 probe** 05523-L04951		**Exon 1**
171	**KCNQ2 probe** 05532-L20535		**Exon 10**
178 *	Reference probe 11571-L12318	16q21	
184	**KCNQ2 probe** 05533-L04961		**Exon 11**
190	Reference probe 12422-L13423	14q24	
197	**KCNQ2 probe** 05525-L04953		**Exon 3**
206 ¥	**KCNQ2 probe** 22012-L05459		**Exon 9**
212	**KCNQ2 probe** 05534-L20314		**Exon 12**
220 « ¬	CHRNA4 probe 06609-L20315		**Downstream**
228	**KCNQ2 probe** 05526-L20316		**Exon 4**
234	**KCNQ2 probe** 05535-L20317		**Exon 13**
241 ¬	TNFRSF6B probe 01966-L20318		**Upstream**
248	**KCNQ2 probe** 05527-L04955		**Exon 5**
256 « +	*SHANK3 probe 14181-L15791*	*22q13*	
265 *	Reference probe 19015-L25096	21q21	
274	**KCNQ2 probe** 06031-L04956		**Exon 6**
284	**KCNQ2 probe** 05537-L20536		**Exon 15**
292 *	Reference probe 10136-L10598	18q11	
301 ¥	**KCNQ2 probe** 07787-L31198		**Exon 7**
314 ¥	**KCNQ2 probe** 07790-L31199		**Exon 16**
325 *	Reference probe 18454-L23630	7q31	
340 ¥	**KCNQ2 probe** 05539-L31026		**Exon 17**
346	**KCNQ2 probe** 07789-L07525		**Exon 14**
352 « ¬	EEF1A2 probe 05920-L01290		**Upstream**
363 *	Reference probe 20535-L28125	1q31	
373	**KCNQ2 probe** 07788-L07524		**Exon 8**
382	**KCNQ2 probe** 07786-L07522		**Exon 2**
391 *	Reference probe 08671-L08683	9q31	

* New in version C2 (from lot C2-0518 onwards).

¥ Changed in version C2 (from lot C2-0518 onwards). Small change in length, 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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

+ The 256 nt SHANK3 probe has been included as an extra warning for incomplete DNA denaturation.

Note: The exon numbering used in this P166-C2 KCNQ2 product description is the exon numbering from the RefSeq transcript NM_172107.3, which is identical to the NG_009004.2 sequence. The exon numbering and NM sequence used is from 02/2018 but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. KCNQ2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*KCNQ2* Exon	Ligation site NM_172107.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
241 ¬	01966-L20318	*TNFRSF6B*		GCGGAGTGGCAG-AAACACCCACCT	206.4 kb
352 « ¬	05920-L01290	*EEF1A2*		GGGGACAGCAAG-TCTGACCCGCCG	16.2 kb
		start codon	*215-217 (exon 1)*		
142 «	05522-L05458	upstream	1725 nt before exon 1	TTCCTTCTGCAT-ACTGAAGTCCTC	2.1 kb
159 «	05523-L04951	Exon 1	441-442	CTTCTACCGCAA-GCTGCAGAATTT	25.5 kb
382	07786-L07522	Exon 2	550-551	CTGTCTGTGTTT-TCCACCATCAAG	1.5 kb
197	05525-L04953	Exon 3	716-717	CCCGGAAACCGT-TCTGTGTGATTG	0.6 kb
228 #	05526-L20316	Exon 4	881-882	AGCTGCTGGGCT-CTGTGGTCTATG	2.2 kb
248	05527-L04955	Exon 5	936-937	CATCGGCTTCCT-TTGTCTCATCCT	2.8 kb
274	06031-L04956	Exon 6	1032-1033	GGTCCCACAGAT-CACGCTGACCAC	1.0 kb
301	07787-L31198	Exon 7	1208-1209	ACTTTGAGAAGA-GGCGGAACCCGG	4.8 kb
373	07788-L07524	Exon 8	1253-1254	CCTGGAGATTCT-ACGCCACCAACC	2.6 kb
206	22012-L05459	Exon 9	32 nt after exon 9	AGCCCTGTGTGT-GTGTGTGGTTCT	2.9 kb
171	05532-L20535	Exon 10	1397-1398	TGGAGCTGCTGA-GGAACCTCAAGA	4.2 kb
184	05533-L04961	Exon 11	4 nt after exon 11	TCTCCAAGGTCA-GTGCCCCCTGCT	4.6 kb
212	05534-L20314	Exon 12	1514-1515	CCGGACGCTCTA-GGTACCGCGGAA	4.5 kb
234	05535-L20317	Exon 13	1530-1531	GAAGGTCAGTTT-GAAAGATCGTGT	1.0 kb
346	07789-L07525	Exon 14	1806-1807	TGTGACCGAGGA-CCTGACCCCGGG	0.6 kb
284	05537-L20536	Exon 15	1877-1878	CCAAGCGGAAGT-TCAAGGAGAGCC	5.1 kb
314	07790-L31199	Exon 16	2088-2089	ACGGCTCGGGAA-GGTGGAGAAGCA	2.0 kb
340	05539-L31026	Exon 17	3007-3008	GCAGTGACCTTT-TACAAAAGTTAT	46.8 kb
		stop codon	*2831-2833 (exon 17)*		
220 « ¬	06609-L20315	*CHRNA4*		TGAAGAAACTCT-TCTCCGGTTACA	

« 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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Note: The exon numbering used in this P166-C2 KCNQ2 product description is the exon numbering from the RefSeq transcript NM_172107.3, which is identical to the NG_009004.2 sequence. The exon numbering and NM sequence used is from 02/2018 but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

P197 KCNQ3	Contains probes for the *KCNQ3*, *CHRNA4*, *EPM2A*, *NHLRC1* (*EPM2B*) and *CHRNB2* genes.
P137 SCN1A	Contains probes for all exons of *SCN1A*.
P267 Dandy walker	Contains probes for the *ZIC1*, *ZIC4* and *VLDLR* genes.
P343 Autism-1	Contains probes for the 15q11, 15q13, 16p11.2 genomic regions and the *SHANK3* gene at 22q13.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P166 KCNQ2

- Grinton BE et al. (2015). Familial neonatal seizures in 36 families: Clinical and genetic features correlate with outcome. *Epilepsia*. 56:1071-80.
- Heron SE et al. (2007). Deletions or duplications in KCNQ2 can cause benign familial neonatal seizures. *J Med Genet*. 44:791-6.
- Kurahashi H et al. (2009). Deletions involving both KCNQ2 and CHRNA4 present with benign familial neonatal seizures. *Neurology*. 73:1214-17.
- Kwong AK et al. (2015). Analysis of mutations in 7 genes associated with neuronal excitability and synaptic transmission in a cohort of children with non-syndromic infantile epileptic encephalopathy. *PLoS One*. 10(5):e0126446.
- Mulley JC and Mefford HC (2011). Epilepsy and the new cytogenetics. *Epilepsia*. 52:423-32.
- Soldovieri MV et al. (2014). Novel *KCNQ2* and *KCNQ3* Mutations in a Large Cohort of Families with Benign Neonatal Epilepsy: First Evidence for an Altered Channel Regulation by Syntaxin-1A. *Hum Mutat*. 35:356-67.
- Zara F et al. (2013). Genetic testing in benign familial epilepsies of the first year of life: Clinical and diagnostic significance. *Epilepsia*. 54:425-36.

P166 Product history	
Version	*Modification*
C2	Six reference probes have been replaced and four probe lengths have been adjusted.
C1	One KCNQ1 probe and several reference probes have been replaced. The 88 and 96 nt control fragments have been replaced and one X-fragment at 100 nt and one Y-fragment at 105 nt have been included (QDX2).
B	Four new KCNQ2 probes and the DNA Denaturation control fragments at 88 and 96 nt have been added.
A	First release.

Implemented changes in the product description

Version C2-01 – 17 July 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Ligation sites of the probes targeting the KCNQ2 gene updated according to new version of the NM_reference sequence.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 10 – 11 January 2017 (55)
- Warning added in Table 1, 265 nt probe 15472-L17312, 292 nt probe 02840-L02271, and 391 nt probe 01337-L02333.
- Various minor textual and layout changes.

Version 09 – 05 November 2015 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes on page 1.

- New references added on page 2.

Version 08 – 15 July 2015 (54)

- Figure based on the use of old MLPA buffer (replaced in December 2012) removed.

Version 07 (48)

- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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