Product Description
SALSA® MLPA® Probemix P217-C1 IGF1R

To be used with the MLPA General Protocol.

Version C1
As compared to version B2, Two target probes have been replaced and two target probes have been added, eight reference probes have been replaced and one reference probe has been added, in addition 16 probe lengths have been adjusted. For complete product history see page 8.

Catalogue numbers:
- **P217-025R:** SALSA MLPA Probemix P217 IGF1R, 25 reactions.
- **P217-050R:** SALSA MLPA Probemix P217 IGF1R, 50 reactions.
- **P217-100R:** SALSA MLPA Probemix P217 IGF1R, 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 P217 IGF1R is a **research use only (RUO)** assay for the detection of deletions or duplications in the *IGF1R, IGFBP3,* and *IGFALS* genes, which are associated with prenatal and postnatal growth failure.

Insulin-like growth factor 1 receptor (IGF1R) is a receptor that binds insulin-like growth factor with a high affinity and plays a critical role in transformation events. Cleavage of the IGF1R precursor generates alpha and beta subunits. It is highly overexpressed in many malignant tissues where it functions as an anti-apoptotic agent by enhancing cell survival. Mutations in the *IGF1R* gene results in IGF1 resistance which may underlie some cases of prenatal and postnatal growth failure.

The *IGFBP3* gene encodes for the insulin-like growth factor binding protein 3 (IGFBP3*)* which is a member of the insulin-like growth factor binding protein (IGFBP) family. The protein forms a ternary complex with insulin-like growth factor acid-labile subunit (IGFALS) and either insulin-like growth factor (IGF) I or II. The IGFs family comprise of peptides that play important roles in mammalian growth and development. IGF1 mediates many of the growth-promoting effects of growth hormone.

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 *IGF1R, IGFBP3,* and *IGFALS* exon numbering used in this P217-C1 IGF1R product description is the exon numbering from the LRG_1055, LRG_68, and NG_011778.1 sequences, respectively. 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 P217-C1 IGF1R contains 45 MLPA probes with amplification products between 127 and 480 nucleotides (nt). This includes 27 probes for the *IGF1R* gene, six probes for the *IGFBP3* gene and three probes for the *IGFALS* gene. 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 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 prenatal and postnatal growth failure. 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. 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 *IGF1R, IGFBP3,* and *IGFALS* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P217 IGF1R.
- 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/. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). 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 *IGF1R* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P217-C1 IGF1R

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*IGF1R*	*IGFBP3*	*IGFALS*
64-105	Control fragments – see table in probemix content section for more information				
127 *	Reference probe 15370-L19110	7q			
136 ¥	**IGFALS probe** 09761-L32574				**Exon 2**
142 «	**IGF1R probe** 13509-L15315		**Exon 1**		
147	**IGF1R probe** 13510-L15316		**Exon 3**		
152	**IGF1R probe** 13511-L15317		**Exon 2**		
160 *	Reference probe 16545-L19036	11q			
165	**IGF1R probe** 07606-L14930		**Exon 17**		
171	**IGF1R probe** 07599-L07284		**Exon 10**		
178	**IGFALS probe** 09759-L16555				**Exon 1**
185	**IGF1R probe** 07603-L07288		**Exon 14**		
193 ¥	**IGF1R probe** 10332-L32439		**Exon 4**		
199	**IGF1R probe** 07609-L10819		**Exon 20**		
207 *	Reference probe 20845-L28863	21q			
214	**IGF1R probe** 07610-L07295		**Exon 21**		
223 *	**IGF1R probe** 23014-L32588		**Exon 16**		
229 ¥	**IGF1R probe** 07597-L32440		**Exon 8**		
236 ¥	**IGFBP3 probe** 07617-L32444			**Exon 5**	
244 ¥	**IGF1R probe** 07598-L32445		**Exon 9**		
250 *	Reference probe 08695-L08707	13q			
260 *	**IGF1R probe** 23015-L32459		**Exon 15**		
268 ¥ «	**IGF1R probe** 13512-L32436		**Exon 1**		
274	**IGF1R probe** 07600-L15322		**Exon 11**		
284	**IGF1R probe** 07611-L15321		**Exon 21**		
292 *	Reference probe 22058-L31018	1p			
303 ¥	**IGF1R probe** 07601-L32446		**Exon 12**		
310	**IGFBP3 probe** 07612-L08916			**Intron 1**	
317	**IGF1R probe** 07602-L07287		**Exon 13**		
328 *	**IGF1R probe** 23017-L32461		**Exon 3**		
337 *	Reference probe 17880-L22139	19q			
346 ¥	**IGF1R probe** 07608-L07293		**Exon 19**		
355	**IGF1R probe** 13513-L15319		**Exon 2**		
366 *	Reference probe 18546-L23322	10q			
373 ¥	**IGFBP3 probe** 07613-L32575			**Exon 2**	
382 ¥	**IGF1R probe** 07595-L32604		**Exon 6**		
391	**IGFBP3 probe** 07615-L09267			**Exon 4**	
407 ¥	**IGF1R probe** 00445-L32448		**Exon 21**		
412 ¥	**IGF1R probe** 07594-L32449		**Exon 5**		
420 *	Reference probe 16155-L18783	6q			
427	**IGFBP3 probe** 07616-L09266			**Exon 5**	
434 *	**IGFALS probe** 23018-L32462				**Exon 2**
442 ¥	**IGF1R probe** 07596-L32450		**Exon 7**		
449 ¥	**IGFBP3 probe** 07614-L32451			**Exon 3**	
463 ¥	**IGF1R probe** 07607-L20423		**Exon 18**		
472 *	**IGF1R probe** 23016-L32460		**Exon 5**		
480 *	Reference probe 21882-L15817	2q			

[a] See section Exon numbering on page 1 for more information.

* New in version C1.
¥ Changed in version C1. 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.

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. P217-C1 probes arranged according to chromosomal location

Table 2a. *IGFBP3*

Length (nt)	SALSA MLPA probe	*IGFBP3* exon[a]	Ligation site NM_001013398.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*133-135 (Exon 1)*		
310	07612-L08916	Intron 1	605 nt after exon 1	CAGAGTGTAGGT-TTGAACTCCGCG	2.8 kb
373	07613-L32575	Exon 2	681-680, reverse	TTAGCATGCCCT-TTCTTGATGATG	0.7 kb
449	07614-L32451	Exon 3	814-815	AAGACACACTGA-ATCACCTGAAGT	1.8 kb
391	07615-L09267	Exon 4	998-999	GAAGGAGGACGT-GCACTGCTACAG	1.3 kb
427	07616-L09266	Exon 5	1342-1343	CTCACCACATGT-TGGTCGAAGCGG	0.7 kb
236	07617-L32444	Exon 5	2027-2028	GCTTGCTGGGGA-GCCCATCCAGGA	
		stop codon	*1024-1026 (Exon 4)*		

Table 2b. *IGF1R*

Length (nt)	SALSA MLPA probe	*IGF1R* exon[a]	Ligation site NM_000875.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*1044-1046 (Exon 1)*		
268 «	13512-L32436	Exon 1	915-916	TTCCTTCGCCCT-TGTTTTTGGAGG	0.3 kb
142 «	13509-L15315	Exon 1	85 nt after exon 1	CCGAGTTGCCAC-CGTCGCAGCTGT	57.8 kb
355	13513-L15319	Exon 2	1153-1154	CGGGCCAGGCAT-CGACATCCGCAA	0.5 kb
152	13511-L15317	Exon 2	1653-1654	AGTACAACTACC-GCTGCTGGACCA	183.1 kb
147	13510-L15316	Exon 3	101 nt before exon 3	GTGATCTGGTGC-TGGTGGTAGCAG	0.3 kb
328	23017-L32461	Exon 3	1869-1870	AGGGCTGGCGCT-GTGTGGACCGTG	5.3 kb
193	10332-L32439	Exon 4	2099-2100	ATGCTCCAAGGA-TGCACCATCTTC	2.8 kb
472	23016-L32460	Exon 5	2281-2282	AGGAGAGGAGCA-GCTAGAAGGGTA	0.1 kb
412	07594-L32449	Exon 5	116 nt after exon 5	AAGAAAGGAGGA-AGCCGCAGTATT	9.0 kb
382	07595-L32604	Exon 6	2332-2333	GAACTTGCAGCA-ACTGTGGGACTG	2.6 kb
442	07596-L32450	Exon 7	2525-2526	GACGTCCTGCAT-TTCACCTCCACC	1.9 kb
229	07597-L32440	Exon 8	2787-2788	CCGTTTACGTCA-AGGCTGTGACCC	2.8 kb
244	07598-L32445	Exon 9	2915-2916	TCGAACTCCTCT-TCTCAGTTAATC	0.8 kb
171	07599-L07284	Exon 10	3141-3140, reverse	GGGGCAGGCGCA-GCAAGGCCCTTT	5.4 kb
274	07600-L15322	Exon 11	3282-3283	TGCAAGTGGCCA-ACACCACCATGT	1.7 kb
303	07601-L32446	Exon 12	3556-3557	TCCTGGGCCAGT-GACCTGGGAGCC	0.7 kb
317	07602-L07287	Exon 13	3722-3723	GGGGCCAAGCTA-AACCGGCTAAAC	5.0 kb
185	07603-L07288	Exon 14	3872-3873	CTGCCCGTCGCT-GTCCTGTTGATC	0.6 kb
260	23015-L32459	Exon 15	3953-3954	CTGGGGAATGGA-GTGCTGTATGCC	4.7 kb
223	23014-L32588	Exon 16	4160-4161	AACGAGGCCGCA-AGCATGCGTGAG	0.4 kb
165	07606-L14930	Exon 17	4313-4312, reverse	AGAGACCGGAGA-TAACTTTTGAGA	4.0 kb
463	07607-L20423	Exon 18	4477-4478	CATGGTAGCCGA-AGATTTCACAGT	3.7 kb
346	07608-L07293	Exon 19	4604-4605	CTCAAGGATGGA-GTCTTCACCACT	5.6 kb
199	07609-L10819	Exon 20	4741-4740, reverse	AGTTGTCTGGCT-TGTCCAGAAGGC	8.8 kb
407	00445-L32448	Exon 21	5148-5149	CGACCTGCTGAT-CCTTGGATCCTG	0.4 kb
214	07610-L07295	Exon 21	5579-5578, reverse	GTGTACAGTGGT-TGGATGGGGCCA	0.2 kb
284	07611-L15321	Exon 21	5804-5805	CATGGGTGAGCA-TGGCAGCTGGTT	
		stop codon	*5145-5147 (Exon 21)*		

Table 2c. *IGFALS*

Length (nt)	SALSA MLPA probe	*IGFALS* exon[a]	Ligation site NM_004970.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*82-84 (Exon 1)*		
178	09759-L16555	Exon 1	282 nt after exon 1, reverse	GCTGTGCTGGGA-GCTGAAGGGTCC	2.0 kb
434	23018-L32462	Exon 2	1100-1101	CCTGGGGCAGCT-TGAGGTGCTCAC	0.9 kb
136	09761-L32574	Exon 2	1970-1971	GACAGGTCCTCA-GTGTCCTCAGGG	
		stop codon	*1897-1899 (Exon 2)*		

[a] See section Exon numbering on page 1 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.

« 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.

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.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P216 GHD mix 1	Contains probes for *GH1*, *PROP1*, *POU1F1*, *GHRHR*, *HESX1*, *LHX3* and *LHX4*.
P262 GHI	Contains probes for *IGF1*, *GHR*, *JAK2* and *STAT5B*.
P026 Sotos	Contains probes for *NSD1*.
P018 SHOX	Contains probes for the *SHOX* gene and several other probes in the PAR region.

References

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P217 product history	
Version	*Modification*
C1	Two target probes have been replaced and two target probes have been added, eight reference probes have been replaced and one reference probe has been added, in addition 16 probe lengths have been adjusted.
B2	Three reference probes have been replaced and the control fragments have been adjusted (QDX2).
B1	Three probes for the *IGFALS* gene, two additional *IGF1R* probes, and four extra control fragments have been added. Furthermore three *IGF1R* probes and four reference probes have been replaced.
A1	First release.

Implemented changes in the product description

Version C1-01 – 06 April 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 *IGFBP3* and *IGF1R* genes updated according to new version of the NM_ reference sequence.

Version 10 – 27 July 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Table 2 restructured.
- Ligation sites of the *IGF1R* gene in Table 2 adjusted.
- Exon numbering of the *IGFALS* gene in Table 2 adjusted.
- Warnings about probes located in strong CpG islands adjusted in Table 1 and Table 2.

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