

Product Description

SALSA® MLPA® Probemix P316-B4 Recessive Ataxias

To be used with the MLPA General Protocol.

Version B4

As compared to version B3, four reference probes have been replaced and one probe length has been adjusted. For complete product history see page 8.

Catalogue numbers:

- **P316-025R:** SALSA MLPA Probemix P316 Recessive Ataxias, 25 reactions.
- **P316-050R:** SALSA MLPA Probemix P316 Recessive Ataxias, 50 reactions.
- **P316-100R:** SALSA MLPA Probemix P316 Recessive Ataxias, 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 P316 Recessive Ataxias is a **research use only (RUO)** assay for the detection of deletions or duplications in the *APTX*, *SETX*, and *FXN* genes, which are associated with Ataxia with oculomotor apraxia type 1 (AOA1), type 2 (AOA2), and Friedreich ataxia (FRDA).

AOA1 is an autosomal recessive neurodegenerative disorder characterized by a childhood onset of slowly progressive cerebellar ataxia, followed by oculomotor apraxia and a severe primary motor peripheral axonal motor neuropathy. AOA1 has been associated with mutations in the *APTX* gene. The *APTX* gene (9 exons) spans ~29 kb of genomic DNA and is located on chromosome 9p13.3, ~33 Mb from the p-telomere. *APTX* encodes the aprataxin protein, which is involved in single-stranded DNA repair.

AOA2 usually has its onset between the ages of three and 30 years and is characterised by cerebellar atrophy, axonal sensorimotor neuropathy, and oculomotor apraxia. Mutations in the *SETX* gene have been associated with AOA2 and amyotrophic lateral sclerosis type 4 (ALS4). *SETX* encodes the senataxin protein, which is suggested to be involved in DNA and RNA processing. The *SETX* gene (26 exons) spans ~94 kb of genomic DNA and is located on chromosome 9q34.13, ~132 Mb from the p-telomere.

FRDA is characterized by slowly progressive ataxia with mean onset between 10 and 15 years of age. FRDA is caused by mutations in the *FXN* gene, leading to reduced expression of the mitochondrial protein frataxin. This deficiency causes degeneration of nervous tissue in the spinal cord, which leads to ataxia. The *FXN* gene (5 exons) spans ~39 kb of genomic DNA and is located on chromosome 9q21.11, ~69 Mb from the p-telomere. Please note that the major cause of Friedreich's ataxia is an expansion of an intronic trinucleotide repeat, which cannot be detected with this P316-B4 Recessive Ataxias probemix.

More information is available at <https://www.ncbi.nlm.nih.gov/books/NBK1456/>, <https://www.ncbi.nlm.nih.gov/books/NBK1154/>, and <https://www.ncbi.nlm.nih.gov/books/NBK1281/>.

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 *APTX*, *SETX*, and *FXN* exon numbering used in this P316-B4 Recessive Ataxias product description is the exon numbering from the NG_012821.2, LRG_268, and LRG_339 sequences. 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 P316-B4 Recessive Ataxias contains 50 MLPA probes with amplification products between 131 and 481 nucleotides (nt). This includes ten probes for the *APTX* gene, one probe for each exon and two probes for exon 2, five probes for the *FXN* gene and 26 probes for the *SETX* gene, one probe for each exon. 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 AOA1, AOA2, or FRDA. 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 *APTX*, *SETX*, and *FXN* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P316 Recessive Ataxias.
- 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 *SETX* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P316-B4 Recessive Ataxias

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a			
		Reference	SETX	APTX	FXN
64-105	Control fragments – see table in probemix content section for more information				
131 ¥	Reference probe 00797-L25925	5q			
137	SETX probe 21575-L11569		Exon 3		
142 *	Reference probe 19948-L27009	10q			
148	APTX probe 10862-L11532			Exon 5	
154 +	FXN probe 10895-L11565			Exon 5	
160	SETX probe 10898-L14358		Exon 17		
166	SETX probe 10904-L11573		Exon 7		
172	SETX probe 10922-L11591		Exon 21		
178 *	Reference probe 10676-L11258	6p			
184	APTX probe 10890-L11560			Exon 9	
189	SETX probe 21577-L30498		Exon 11		
193	APTX probe 10860-L30496			Exon 3	
198	SETX probe 10919-L14357		Exon 19		
202	SETX probe 10912-L14356		Exon 14		
208	FXN probe 10893-L11563			Exon 2	
214	APTX probe 10888-L11558			Exon 7	
219	SETX probe 10905-L11574		Exon 8		
225	SETX probe 10902-L14355		Exon 5		
229	APTX probe 10889-L11559			Exon 8	
234	FXN probe 10891-L11561			Exon 1	
239	SETX probe 10914-L13898		Exon 15		
246	SETX probe 10925-L14354		Exon 23		
254	SETX probe 13128-L14348		Exon 13		
258	FXN probe 10894-L13901			Exon 3	
265	SETX probe 10918-L11587		Exon 18		
271 *	Reference probe 16225-L32730	16q			
277	SETX probe 19811-L30788		Exon 24		
285	SETX probe 10897-L13900		Exon 1		
293	SETX probe 21576-L30499		Exon 4		
301	SETX probe 10921-L11590		Exon 20		
310	APTX probe 10863-L11533			Exon 6	
319	SETX probe 10910-L11579		Exon 12		
328	APTX probe 10857-L11527			Exon 1	
337	SETX probe 10915-L11584		Exon 16		
346	Reference probe 08024-L07805	11q			
355	SETX probe 10927-L14352		Exon 25		
364	APTX probe 10861-L14351			Exon 4	
373	SETX probe 10907-L11576		Exon 10		
382	Reference probe 17429-L27885	8p			
391	SETX probe 12776-L11572		Exon 6		
400	SETX probe 12777-L11598		Exon 26		
409	SETX probe 12747-L13841		Exon 2		
417	APTX probe 14088-L15687			Exon 2	
427	SETX probe 13129-L14349		Exon 22		
436	Reference probe 04279-L23590	12q			
445 *	Reference probe 16286-L18578	13q			
454	APTX probe 14089-L15688			Exon 2	
463	SETX probe 14128-L15732		Exon 9		
472	FXN probe 14129-L15733			Exon 4	
481	Reference probe 09772-L10187	15q			

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

* New in version B4.

‡ Changed in version B4. Minor alteration, no change in sequence detected.

+ This probe may be influenced by a small nonspecific peak. Please take extra care when interpreting results from this probe. Copy number changes detected by only a single probe always require confirmation by another method.

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. P316-B4 probes arranged according to chromosomal location

Table 2a. *SETX*

Length (nt)	SALSA MLPA probe	<i>SETX</i> exon ^a	Ligation site NM_015046.7	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	185-187 (Exon 3)		
285	10897-L13900	Exon 1	36-37	GGCCCGGTATGG-AGGTGGGCTAGA	1.3 kb
409	12747-L13841	Exon 2	143-144	CTAAGCCCAGCT-GAGACGATCACT	4.3 kb
137	21575-L11569	Exon 3	220-221	CCAGGTGGTGCT-TCCACCATTGAC	3.0 kb
293	21576-L30499	Exon 4	483-484	GCCACTGTTTGA-CATCACTGGGCA	3.6 kb
225	10902-L14355	Exon 5	615-616	GGAACAAGCCAA-TTGCTCCTTTCA	6.3 kb
391	12776-L11572	Exon 6	703-704	TGGGCTATCTTG-ACTGCAAGAAAC	1.8 kb
166	10904-L11573	Exon 7	933-934	CATTCTTGAGGA-ACAAGCCATGGA	3.3 kb
219	10905-L11574	Exon 8	1090-1091	CGCCTTGGATCT-AAGGTCTGGGGT	0.3 kb
463	14128-L15732	Exon 9	1222-1223	GAGTCTATTTG-GATGATATGGTG	0.8 kb
373	10907-L11576	Exon 10	1460-1461	AGGATTTGGGTG-TGGCTTACATAG	18.5 kb
189	21577-L30498	Exon 11	5488-5489	AACTCTCAAAT-AGAGAGAATTTTC	11.2 kb
319	10910-L11579	Exon 12	5725-5726	TTTCGCCGCACG-TCAGTCAGTAAG	2.4 kb
254	13128-L14348	Exon 13	5861-5862	AGTTGAAAGCCA-TGTCTCTGTTGG	1.3 kb
202	10912-L14356	Exon 14	6130-6131	CGTCTACTGACA-GAGGTAGGTATG	0.9 kb
239	10914-L13898	Exon 15	6160-6161	CATTCAGACGAA-AACTCCAATGCC	7.4 kb
337	10915-L11584	Exon 16	6351-6352	TAATAGTGAGGT-TCTAAAGTTTCCAG	0.3 kb
160	10898-L14358	Exon 17	6483-6484	GCAGCGAGCTCT-ATGCCGAGGTGG	1.8 kb
265	10918-L11587	Exon 18	6538-6539	AACATTTCCAAA-GTTTCTAAGGAA	3.1 kb
198	10919-L14357	Exon 19	6646-6647	ATCTGCTGCACG-TTGAGCACAAGT	1.8 kb
301	10921-L11590	Exon 20	6782-6783	TCCATCGCTGCA-ATAAGCTCATCC	3.3 kb
172	10922-L11591	Exon 21	6891-6892	CTTCTGCAGACT-GCTGGAAGAGAA	1.1 kb
427	13129-L14349	Exon 22	7073-7074	CATTTCCAGCCAT-ACCTTGTGTTTG	1.8 kb
246	10925-L14354	Exon 23	7236-7237	TTACAAGGCCCA-GAAGACGATGAT	3.5 kb
277	19811-L30788	Exon 24	7312-7313	ACTGTGGATGCA-TTCCAGGGTCCG	2.1 kb
355	10927-L14352	Exon 25	7412-7413	AGAGATTGAATG-TCACCATCACAC	4.9 kb
400	12777-L11598	Exon 26	7687-7688	GGATTTGCCAAG-ACATCTGTTGCT	
		<i>stop codon</i>	8216-8218 (Exon 26)		

Table 2b. *APTX*

Length (nt)	SALSA MLPA probe	<i>APTX</i> exon ^a	Ligation site NM_175073.2	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	186-188 (Exon 3)		
328	10857-L11527	Exon 1	68-69	CCGTCTCCGACT-TCTGGAGGTAAG	4.2 kb
417	14088-L15687	Exon 2	83 nt before exon 2	CTCTAGCATTGT-GCTTCCACCCTT	0.3 kb
454	14089-L15688	Exon 2	75 nt after exon 2	TTATTTCCAGTA-CTAGAATGGCAC	7.3 kb
193	10860-L30496	Exon 3	204-205	GGGTGTGCTGGT-TGGTGAGACAGG	1.8 kb
364	10861-L14351	Exon 4	334-335	GTTGAAAGCAGA-GTGTAACAAGGG	0.3 kb
148	10862-L11532	Exon 5	415-416	TGGGAAGGACCA-AGAGGTGAAGCT	1.8 kb
310	10863-L11533	Exon 6	697-698	GAGTCAAGGCTT-GAAGATTTCTAT	1.2 kb
214	10888-L11558	Exon 7	814-815	GTGGACCTCCAT-TTCCAGTCTGAA	10.2 kb
229	10889-L11559	Exon 8	978-979	TTCATGTGATCA-GCCAGGATTTTG	1.0 kb

184	10890-L11560	Exon 9	1159-1160	GTGCCAGCAGCT-GCTGCCTTCCAT	
		stop codon	1212-1214 (Exon 9)		

Table 2c. FXN

Length (nt)	SALSA MLPA probe	FXN exon ^a	Ligation site NM_000144.5	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		start codon	32-34 (Exon 1)		
234	10891-L11561	Exon 1	232 nt before exon 1	CAGCTCCAAGT-TCCTCCTGTTTA	10.9 kb
208 #	10893-L11563	Exon 2	274-275	TTGAGGAAATCT-GGAACTTTGGGC	6.7 kb
258 #	10894-L13901	Exon 3	328-329	GAAAGACTAGCA-GAGGAAACGCTG	11.9 kb
472	14129-L15733	Exon 4	24 nt after exon 4	GTTCAGAAGTCA-ACATATGTAATT	8.3 kb
154 #+	10895-L11565	Exon 5	1259-1260	CTGGGTTGTCCA-GGGAGACCTAGT	
		stop codon	662-664 (Exon 5)		

^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.

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.

+ This probe may be influenced by a small nonspecific peak. Please take extra care when interpreting results from this probe. Copy number changes detected by only a single probe always require confirmation by another method.

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

P041 ATM-1/P042 ATM-2 Contains probes for the *ATM* gene for the diagnosis of Ataxia-Telangiectasia.

References

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Selected publications using SALSA MLPA Probemix P316 Recessive Ataxias

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P316 product history	
<i>Version</i>	<i>Modification</i>
B4	Four reference probes have been replaced and one probe length has been adjusted.
B3	Five reference probes have been replaced. In addition, several probe lengths have been adjusted.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	The APTX exon 2 probe has been replaced, and one APTX, one SETX and one FXN probe have been added. Probes are now present for each exon of these genes.
A1	First release.

Implemented changes in the product description
<p>Version B4-01 – 27 September 2021 (04P)</p> <ul style="list-style-type: none"> - 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 SETX and FXN genes updated according to new version of the NM_ reference sequence. <p>Version B3-01 – 30 April 2018 (01P)</p> <ul style="list-style-type: none"> - 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). - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. <p>Version 12 – 26 September 2016 (55)</p> <ul style="list-style-type: none"> - Warning added on probe 10895-L11565. - The NM_ reference sequences of the APTX and FXN genes in Tables 2b and 2c have been adjusted according to the NM_ sequence used in the RefSeqGene project. - Small textual changes page 1 <p>Version 11 – 10 December 2015 (55)</p> <ul style="list-style-type: none"> - 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. - New references added. <p>Version 10 - 07 August 2015 (48)</p> <ul style="list-style-type: none"> - Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed.

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