Product Description SALSA® MLPA® Probemix P316-B3 Recessive Ataxias

To be used with the MLPA General Protocol.

Version B3. As compared to version B2, five reference probes have been replaced. In addition, several probe lengths have been adjusted. For complete product history see page 6.

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, 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 P316 Recessive Ataxias is a **research use only (RUO)** assay for the detection of deletions or duplications in *APTX*, *SETX*, and *FXN* genes, which are associated with Ataxia with oculomotor apraxia type 1 (AOA1), type 2 (AOA2), and Friedreich ataxia (FRDA).

Ataxia with oculomotor apraxia type 1 (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 9p21.1, ~33 Mb from the p-telomere. *APTX* encodes the aprataxin protein, which is involved in single-stranded DNA repair.

AOA type 2 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.

Friedreich ataxia (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-B3 Recessive Ataxias probemix.

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 P316-B3 Recessive Ataxias contains 50 MLPA probes with amplification products between 128 and 481 nt. This includes ten probes for the *APTX* gene, five probes for the *FXN* gene, and 26 probes for the *SETX* gene. In addition, nine reference probes are included and detect nine 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 Ataxia. 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. 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 *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. 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 *SETX* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P316-B3 Recessive Ataxias

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)			
		Reference	*SETX*	*APTX*	*FXN*
64-105	Control fragments – see table in probemix content section for more information				
128	Reference probe 00797-L00093	5q31			
137 ¥	**SETX probe** 21575-L11569		**Exon 3**		
142 *	Reference probe 13225-L14558	1p21			
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 10509-L11062	7q34			
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**		
270 *	Reference probe 10270-L14339	6q14			
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	11q24			
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	8p21			
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	12q12			
445	Reference probe 12758-L13874	4q12			
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	15q21			

* New in version B3 (from lot B3-0318 onwards).
¥ Changed in version B3 (from lot B3-0318 onwards). Small change in length, 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.

Table 2. P316-B3 probes arranged according to chromosomal location

Table 2a. *SETX*

Length (nt)	SALSA MLPA probe	SETX Exon	Ligation site NM_015046.5	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*184-186 (exon 3)*		
285	10897-L13900	Exon 1	35-36	GGCCCGGTATGG-AGGTGGGCTAGA	1.3 kb
409	12747-L13841	Exon 2	142-143	CTAAGCCCAGCT-GAGACGATCACT	4.3 kb
137	21575-L11569	Exon 3	219-220	CCAGGTGGTGCT-TCCACCATTGAC	3.0 kb
293	21576-L30499	Exon 4	482-483	GCCACTGTTTGA-CATCACTGGGCA	3.6 kb
225	10902-L14355	Exon 5	614-615	GGAACAAGCCAA-TTGCTCCTTTCA	6.3 kb
391	12776-L11572	Exon 6	702-703	TGGGCTATCTTG-ACTGCAAGAAAC	1.8 kb
166	10904-L11573	Exon 7	932-933	CATTCTTGAGGA-ACAAGCCATGGA	3.3 kb
219	10905-L11574	Exon 8	1089-1090	CGCCTTGGATCT-AAGGTCTGGGGT	0.3 kb
463	14128-L15732	Exon 9	1221-1222	GAGTCCTATTTG-GATGATATGGTG	0.8 kb
373	10907-L11576	Exon 10	1459-1460	AGGATTTGGGTG-TGGCTTACATAG	18.5 kb
189	21577-L30498	Exon 11	5487-5488	AACTCTCCAAAT-AGAGAGAATTTC	11.2 kb
319	10910-L11579	Exon 12	5724-5725	TTTCGCCGCACG-TCAGTCAGTAAG	2.4 kb
254	13128-L14348	Exon 13	5860-5861	AGTTGAAAGCCA-TGTCTCTGTTGG	1.3 kb
202	10912-L14356	Exon 14	6129-6130	CGTCTACTGACA-GAGGTAGGTATG	0.9 kb
239	10914-L13898	Exon 15	6159-6160	CATTCAGACGAA-AACTCCAATGCC	7.4 kb
337	10915-L11584	Exon 16	6350-6351	TAATAGTGAGGT-TCTAAAGTTCAG	0.3 kb
160	10898-L14358	Exon 17	6482-6483	GCAGCGAGCTCT-ATGCCGAGGTGG	1.8 kb
265	10918-L11587	Exon 18	6537-6538	AACATTTCCAAA-GTTTCTAAGGAA	3.1 kb
198	10919-L14357	Exon 19	6645-6646	ATCTGCTGCACG-TTGAGCACAAGT	1.8 kb
301	10921-L11590	Exon 20	6781-6782	TCCATCGCTGCA-ATAAGCTCATCC	3.3 kb
172	10922-L11591	Exon 21	6890-6891	CTTCTGCAGACT-GCTGGAAGAGAA	1.1 kb
427	13129-L14349	Exon 22	7072-7073	CATTTCAGCCAT-ACCTTGTGTTTG	1.8 kb
246	10925-L14354	Exon 23	7235-7236	TTACAAGGCCCA-GAAGACGATGAT	3.5 kb
277	19811-L30788	Exon 24	7311-7312	ACTGTGGATGCA-TTCCAGGGTCGG	2.1 kb
355	10927-L14352	Exon 25	7411-7412	AGAGATTGAATG-TCACCATCACAC	4.9 kb
400	12777-L11598	Exon 26	7686-7687	GGATTTGCCAAG-ACATCTGTTGCT	
		stop codon	*8215-8217 (exon 26)*		

Table 2b. *APTX*

Length (nt)	SALSA MLPA probe	APTX Exon	Ligation site NM_175073.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*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	TTATTCCCAGTA-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	Ligation site NM_000144.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*221-223 (exon 1)*		
234	10891-L11561	Exon 1	43 nt before exon 1	CAGCTCCCAAGT-TCCTCCTGTTTA	10.9 kb
208 #	10893-L11563	Exon 2	463-464	TTGAGGAAATCT-GGAACTTTGGGC	6.7 kb
258 #	10894-L13901	Exon 3	517-518	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	1448-1449	CTGGGTTGTCCA-GGGAGACCTAGT	
		stop codon	*851-853 (exon 5)*		

+ 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.
# 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 P316-B3 Recessive Ataxias product description is the exon numbering from the RefSeq transcript NM_015046.5, which is identical to the LRG_268 sequence. The exon numbering from the RefSeq transcripts NM_175073.2 and NM_000144.4 are identical to the NG_012821.1 and NG_008845.2 sequence, respectively. The exon numbering and NM sequences used are from 03/2018, 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

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

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.
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Selected publications using SALSA MLPA Probemix P316 Recessive Ataxias

- Anheim M et al. (2012). Exonic Deletions of FXN and Early-Onset Friedreich Ataxia. *Arch Neurol.* 69:912-916.
- Deutsch EC et al. (2010). A rapid, noninvasive immunoassay for frataxin: Utility in assessment of Friedreich ataxia. *Mol Genet Metab*. 101:238-245.
- Hoffman-Zacharska D et al. (2016). Friedreich ataxia is not only a GAA repeat expansion disorder: implications for molecular testing and counselling. *J Appl Genet.* 57:349-355.
- Nanetti L et al. (2013). SETX mutations are a frequent genetic cause of juvenile and adult onset cerebellar ataxia with neuropathy and elevated serum alpha-fetoprotein. *Orphanet J Rare Dis.* 8:123.
- Van den Ouweland AMW et al. (2012). Complete FXN Deletion in a Patient with Friedreich's Ataxia. *Genet Test Mol Biomarkers* 16:1015-1018.
- Van Minkelen R et al. (2015). Complete APTX deletion in a patient with ataxia with oculomotor apraxia type 1. *BMC Med Genet.* 16:61.
- Wedding IM et al. (2015). Friedreich ataxia in Norway–an epidemiological, molecular and clinical study. *Orphanet J Rare Dis* 10.1: 108.

P316 Product history	
Version	*Modification*
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
Version B3-01 – 30 April 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).

- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 12 – 26 September 2016 (55)
- 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

Version 11 – 10 December 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.
- New references added.

Version 10 - 07 August 2015 (48)
- Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed.

Version 09 (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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