Product Description
SALSA® MLPA® Probemix P432-A2 MYH9

To be used with the MLPA General Protocol.

Version A2
As compared to version A1, three reference probes have been replaced. For complete product history see page 9.

Catalogue numbers
- **P432-025R:** SALSA® MLPA® Probemix P432 MYH9, 25 reactions.
- **P432-050R:** SALSA® MLPA® Probemix P432 MYH9, 50 reactions.
- **P432-100R:** SALSA® MLPA® Probemix P432 MYH9, 100 reactions.

SALSA® MLPA® Probemix P432 MYH9 (hereafter: P432 MYH9) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

P432 MYH9 can be used in combination with:

- SALSA® Binning DNA SD035 (Cat. No: SD035)

Volumes and ingredients

Volumes			Ingredients
P432-025R	**P432-050R**	**P432-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

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
SALSA® MLPA® Probemix P432 MYH9 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MYH9* gene, which is associated with *MYH9*-related disorder (MYH9RD). This probemix can also be used to detect the presence of the c.287C>T (p.S96L) and c.2104C>T (p.R702C) point mutations as well as the wild type sequence of the c.4270G>C/A/T (p.D1424H/N/Y) mutation.

The *MYH9* gene is located on chromosome 22q12.3 and encodes the non-muscle myosin (heavy chain, type II-A). *MYH9*-related disorder (MYH9RD) is an autosomal dominant platelet disorder characterized by macrothrombocytopenia and thrombocytopenia. The complex phenotype of MYH9RD may include bleeding problems, hearing loss, renal disease, and cataracts. Epstein syndrome, Fechtner syndrome, May-Hegglin anomaly and Sebastian syndrome, previously described as distinct disorders, represent some of the different clinical presentations of MYH9RD.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK2689/.

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

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 *MYH9* exon numbering used in this P432-A2 MYH9 product description is the exon numbering from the LRG_567 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 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
P432-A2 MYH9 contains 53 MLPA probes with amplification products between 128 and 500 nucleotides (nt). This includes 42 probes for the *MYH9* gene. Furthermore, this probemix also contains two probes specific for the c.287C>T and the c.2104C>T mutation, which will only generate a signal when the respective mutation is present. One probe that will only detect the wild type sequence of the c.4270G>C/A/T mutation is also included. In addition, eight 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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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. Note that the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.

Required specimens
Extracted DNA, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 MYH9RK. 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.

SALSA® Binning DNA SD035
The SALSA® Binning DNA SD035 provided with this probemix can be used for binning of all probes including the two mutation-specific probes (MYH9 probe 18610-SP0671-L24707 for the c.2104C>T mutation, and MYH9 probe 18616-SP0675-L24711 for the c.287C>T mutation). SD035 is a mixture of human female genomic DNA from healthy individuals and a titrated amount of plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD035 in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net. Furthermore, binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). For further details, please consult the SD035 product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

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 *MYH9* gene are small (point) mutations. Most of which will not be detected by using SALSA MLPA Probemix P432 MYH9, next to the ones detected by the two mutation and wild type specific probes included in this Probemix.
- 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.

***MYH9* mutation database**

https://databases.lovd.nl/shared/genes/MYH9. 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 *MYH9* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P432-A2 MYH9

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*MYH9*
64-105	Control fragments – see table in probemix content section for more information		
128	Reference probe S0584-L22851	20p	
132	Reference probe 00797-L21698	5q	
137	**MYH9 probe** 18602-L23959		**Exon 11**
142	**MYH9 probe** 18603-L23960		**Exon 19**
148 «	**MYH9 probe** 18604-L23961		**Exon 1**
154	**MYH9 probe** 18605-L23962		**Exon 18**
160	**MYH9 probe** 18606-L23963		**Exon 29**
166 Ж	**MYH9 probe** 18607-SP0669-L24705		**Exon 33**
172	Reference probe 10922-L25079	9q	
178 Ж	**MYH9 probe** 18608-SP0670-L24706		**Exon 22**
184	**MYH9 probe** 18609-L24883		**Exon 41**
190 § Ж	**MYH9 probe** 18610-SP0671-L24707		**Exon 17** c.2104C>T
197	**MYH9 probe** 18611-L23968		**Exon 7**
202	**MYH9 probe** 18612-L24708		**Exon 38**
208 Ж	**MYH9 probe** 18613-SP0672-L24709		**Exon 14**
214 Ж	**MYH9 probe** 18614-SP0673-L23971		**Exon 2**
220 Ж	**MYH9 probe** 18615-SP0674-L24712		**Exon 37**
226 § Ж	**MYH9 probe** 18616-SP0675-L24711		**Exon 2** c.287C>T
232 Ж	**MYH9 probe** 18617-SP0676-L23974		**Exon 27**
241 Ж	**MYH9 probe** 18618-SP0677-L23975		**Exon 31**
250	**MYH9 probe** 18619-L23976		**Exon 24**
256 Ж	**MYH9 probe** 18620-SP0678-L23977		**Exon 25**
262	Reference probe 12434-L13435	14q	
268 Ж	**MYH9 probe** 18621-SP0679-L25037		**Exon 6**
274 Ж	**MYH9 probe** 18622-SP0680-L24888		**Exon 17**
283	**MYH9 probe** 18623-L23980		**Exon 39**
290	**MYH9 probe** 18624-L23981		**Exon 5**
296 Ж	**MYH9 probe** 18625-SP0681-L23982		**Exon 12**
304	Reference probe 16436-L18889	18q	
310 ∞ Ж	**MYH9 probe** 18626-SP0682-L24596		**Exon 31** c.4270G>C/A/T
321 Ж Ø	**MYH9 probe** 18627-SP0683-L23984		Intron 1
330 Ж	**MYH9 probe** 18628-SP0684-L23985		**Exon 30**
337	**MYH9 probe** 18629-L24886		**Exon 20**
346	**MYH9 probe** 18630-L23987		**Exon 13**
355 Ж	**MYH9 probe** 18631-SP0685-L24887		**Exon 10**
364 *	Reference probe 11592-L12339	16q	
370	**MYH9 probe** 18632-L24598		**Exon 40**
379	**MYH9 probe** 18633-L23990		**Exon 4**
387	**MYH9 probe** 18634-L24599		**Exon 26**
395	**MYH9 probe** 18635-L23992		**Exon 15**
402	**MYH9 probe** 18636-L24600		**Exon 16**
409 Ж	**MYH9 probe** 18637-SP0686-L24601		**Exon 9**
419 *	Reference probe 17462-L23159	12p	
427 Ж	**MYH9 probe** 18638-SP0687-L24602		**Exon 3**
432 Ж	**MYH9 probe** 18639-SP0688-L23996		**Exon 35**
440	**MYH9 probe** 18641-L24603		**Exon 23**
449	**MYH9 probe** 18642-L23999		**Exon 8**
459	**MYH9 probe** 18643-L24000		**Exon 32**
465	**MYH9 probe** 18644-L24001		**Exon 21**
472 Ж	**MYH9 probe** 18645-SP0689-L24604		**Exon 41**
483	**MYH9 probe** 18646-L24003		**Exon 34**

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*MYH9*
490	**MYH9 probe** 18647-L24004		**Exon 28**
500 *	Reference probe 09682-L22509	3p	

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

* New in version A2.
§ Mutation-specific probe. These probes will only generate a signal when the c.287C>T (226 nt) or c.2104C>T (190 nt) mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
∞ Wild type sequence detected. A lowered probe signal can be due to a c.4270G>C/A/T mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
« 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.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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. *MYH9* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*MYH9* exon[a]	Ligation site[b] NM_002473.6	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*181-183 (Exon 2)*		
148 «	18604-L23961	Exon 1	8-9	GCGCGCAGATCA-CCGCGGTTCCTG	22.9 kb
321 Ø Ж	18627-SP0683-L23984	Intron 1	Both 15.9 kb before exon 2	AAAGATCATGGG-26 nt spanning oligo-CGAGCCCAGCTC	15.9 kb
214 Ж	18614-SP0673-L23971	Exon 2	282-281 and 252-251, reverse	TTGTCGGAAGGC-30 nt spanning oligo-GCCTGGGCCAGC	0.2 kb
226 § Ж	18616-SP0675-L24711	Exon 2	1 nt after exon 2 and 467-466, reverse	GGCAGCCACTTA-48 nt spanning oligo-AGGCTTCGTTGA	7.5 kb
427 Ж	18638-SP0687-L24602	Exon 3	576-577 and 607-608	ATCTACTCTGAA-31 nt spanning oligo-GGCACGAGATGC	13.9 kb
379	18633-L23990	Exon 4	684-685	CGAGAAGATCAA-TCCATCTTGTGC	0.9 kb
290	18624-L23981	Exon 5	750-749, reverse	ACGTACGCCAGA-TACTGGATGACC	4.2 kb
268 Ж	18621-SP0679-L25037	Exon 6	822-823 and 846-847	CTGCTGCAGGCC-24 nt spanning oligo-AACGCCAAGACC	0.7 kb
197	18611-L23968	Exon 7	944-945	AGCCAACATTGA-GACTTGTATCCT	1.0 kb
449	18642-L23999	Exon 8	1041-1042	GCTGGAGAGCAC-CTGAAGAGTGAG	0.6 kb
409 Ж	18637-SP0686-L24601	Exon 9	1159-1160 and 5 nt after exon 9	TGGAGGCCATGA-38 nt spanning oligo-GGGCTGGCCCTC	0.7 kb
355 Ж	18631-SP0685-L24887	Exon 10	1251-1252 and 1281-1282	TTCAAGAAGGAG-30 nt spanning oligo-AACACAGGTAAC	1.2 kb
137	18602-L23959	Exon 11	1296-1297	TTAGCTGCCCAA-AAGGTGTCCCAT	1.7 kb
296 Ж	18625-SP0681-L23982	Exon 12	1453-1454 and 1483-1484	CCTATGAGCGGA-30 nt spanning oligo-AGGCTCTGGACA	2.3 kb
346	18630-L23987	Exon 13	1569-1570	CAGCTGAACTCG-TTTGAGCAGCTG	2.2 kb
208 Ж	18613-SP0672-L24709	Exon 14	1835-1836 and 1858-1859	GGAGCAAGGCAC-23 nt spanning oligo-AGCAGCTGAAGG	2.8 kb
395	18635-L23992	Exon 15	2005-2006	ACAAGTTTGTCT-CGGAGCTGTGGA	2.8 kb
402	18636-L24600	Exon 16	2157-2156, reverse	TTCCTCAGCGTA-GCCATCAGCTTG	0.5 kb
190 § Ж	18610-SP0671-L24707	Exon 17	2331-2330 and 2284-2283, reverse	CACCTCTGCCGA-47 nt spanning oligo-GATGCCCTCGAG	0.2 kb
274 Ж	18622-SP0680-L24888	Exon 17	145 nt and 175 nt after exon 17	GGCCAGCTCTTC-30 nt spanning oligo-GGAAAGGAGACT	0.6 kb

Length (nt)	SALSA MLPA probe	*MYH9* exon[a]	Ligation site[b] NM_002473.6	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
154	18605-L23962	Exon 18	2347-2348	TCAGATATGAGA-TCCTGACTCCAA	1.1 kb
142	18603-L23960	Exon 19	2569-2570	ACCTGGCCAGGA-AGTGAGTCCCGG	1.3 kb
337	18629-L24886	Exon 20	20 nt before exon 20	GCTCCGCGCTGA-CCATGTTCCTCA	1.0 kb
465	18644-L24001	Exon 21	42 nt before exon 21	CAGAGGCGGGCA-TCTTCCAGCCGA	0.8 kb
178 Ж	18608-SP0670-L24706	Exon 22	2986-2987 and 3015-3016	AGCACCTGCAGG-29 nt spanning oligo-CAGGTTCCTGCC	0.6 kb
440	18641-L24603	Exon 23	11 nt before exon 23	ACCGTGTCTCTG-GCCCCCGGCAGG	1.3 kb
250	18619-L23976	Exon 24	3192-3193	ATAGCTGAGTTC-ACCACCAACCTC	2.0 kb
256 Ж	18620-SP0678-L23977	Exon 25	3304-3305 and 3330-3331	GGGAGGAGAAGC-26 nt spanning oligo-CGGAAGCTGGAG	1.3 kb
387	18634-L24599	Exon 26	3479-3480	TGCCCAGAAGAA-CATGGCCCTCAA	0.7 kb
232 Ж	18617-SP0676-L23974	Exon 27	3763-3764 and 3790-3791	GGCAGAAGCACT-27 nt spanning oligo-AGCTGGAGCAGA	0.6 kb
490	18647-L24004	Exon 28	11 nt before exon 28	GTCTTTGCTTTC-TTCCCTGCTAGG	0.5 kb
160	18606-L23963	Exon 29	4049-4050	CGGGCTTCTCAG-CCAGTCCGACAG	0.4 kb
330 Ж	18628-SP0684-L23985	Exon 30	4150-4151 and 4178-4179	AGAACCGGCAGA-28 nt spanning oligo-GGTGGAGGACGA	1.2 kb
241 Ж	18618-SP0677-L23975	Exon 31	4318-4319 and 4346-4347	GTGTGGGGTGCC-28 nt spanning oligo-GCTCCAGAAGGA	0.1 kb
310 ∞ Ж	18626-SP0682-L24596	Exon 31	4450-4451 and 4483-4484	AGGAGCTGGAC**G**-33 nt spanning oligo-GCGCGTGCAACC	2.7 kb
459	18643-L24000	Exon 32	4544-4545	GGAGGAGAAGAC-CATCTCTGCCAA	0.4 kb
166 Ж #	18607-SP0669-L24705	Exon 33	4833-4832 and 4805-4804, reverse	TTGGCATCTTCG-28 nt spanning oligo-CCAGCTGCGTCT	0.4 kb
483	18646-L24003	Exon 34	8 nt before exon 34	GTTGATGTTCCT-TCCCCCAGGTGC	1.6 kb
432 Ж	18639-SP0688-L23996	Exon 35	49 nt and 20 nt before exon 35	GAGGGTTGGGGT-29 nt spanning oligo-AGCCTCTGTCCC	1.1 kb
	No probe ¤	Exon 36			
220 Ж	18615-SP0674-L24712	Exon 37	30 nt and 3 nt before exon 37	GTCCCCAGGGTA-27 nt spanning oligo-CAGAGCCCTGGC	0.6 kb
202 #	18612-L24708	Exon 38	5594-5595	CAAGTCCAAGTA-CAAGGCCTCCAT	0.7 kb
283	18623-L23980	Exon 39	5736-5737	CTGCAGGTGGAT-GACGAGCGGAGG	0.2 kb
370	18632-L24598	Exon 40	5797-5798	CTACCCGCCTGA-AGCAGCTCAAGC	1.5 kb
184	18609-L24883	Exon 41	6023-6024	CGAAGAGGTAGA-TGGCAAAGCGGA	0.6 kb
472 Ж	18645-SP0689-L24604	Exon 41	6578-6579 and 6617-6618	GGCCTCCCACTC-39 nt spanning oligo-CTCCACCTCTGT	
		stop codon	*6061-6063 (Exon 41)*		

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

§ Mutation-specific probe. These probes will only generate a signal when the c.287C>T (226 nt) or c.2104C>T (190 nt) mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
∞ Wild type sequence detected. A lowered probe signal can be due to a c.4270G>C/A/T mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
« 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.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
¤ This exon is not targeted by this probemix.
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.

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 products
For related products, see the product page on our website.

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.

P432 product history	
Version	*Modification*
A2	Three reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version A2-02 – 09 May 2025 (05P) - Product description rewritten and adapted to a new template. - SD035 content has changed from synthetic DNA to plasmid DNA. Version A2-01 – 09 December 2020 (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 name gene updated according to new version of the NM_ reference sequence. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

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