Instructions for Use

SALSA® MLPA® Probemix P034-C1 DMD Mix 1 and SALSA® MLPA® Probemix P035-C1 DMD Mix 2

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

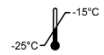
Visit the SALSA® MLPA® Probemix P034 DMD Mix 1 and SALSA® MLPA® P035 DMD Mix 2 product pages on our website to find the Certificate of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P034 DMD Mix 1**
Version	C1
Catalogue numbers	P034-025R (25 reactions) P034-050R (50 reactions) P034-100R (100 reactions)
Basic UDI-DI	872021148P0345M
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Product Name	**SALSA® MLPA® Probemix P035 DMD Mix 2**
Version	C1
Catalogue numbers	P035-025R (25 reactions) P035-050R (50 reactions) P035-100R (100 reactions)
Basic UDI-DI	872021148P0355P
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening 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.

Regulatory Status	
IVD	EUROPE CE 2797 COLOMBIA COSTA RICA ISRAEL MOROCCO
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information: www.mrcholland.com	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, the Netherlands
E-mail	info@mrcholland.com (information & technical questions); order@mrcholland.com (orders)
Phone	+31 888 657 200

Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

P034 version C1. As compared to version B2, four target probes have been replaced and a new target probe for exon 1 has been added. Two reference probes have been redistributed and one reference probe has been added. Two target probes have been adjusted in length.

P035 version C1. As compared to version B1, five target probes have been replaced. Three probes have been adjusted in length and one reference probe has been added.

1. Intended Purpose

The SALSA MLPA Probemixes P034 DMD Mix 1 and P035 DMD Mix 2 are in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assays[2] for the detection of deletions or duplications in the *DMD* gene in genomic DNA isolated from human peripheral whole blood specimens, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or fetal blood. P034 DMD Mix 1 and P035 DMD Mix 2 are intended to confirm a potential cause for and clinical diagnosis of Duchenne muscular dystrophy (DMD) or Becker muscular dystrophy (BMD), for molecular genetic testing of at-risk family members, and for carrier screening.

Copy number variations (CNVs) detected with P034 DMD Mix 1 and P035 DMD Mix 2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *DMD* gene are CNVs, however point mutations can occur which will not be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

These devices are not intended to be used for standalone diagnostic purposes, pre-implantation, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of DMD or BMD. • As all probes in P034 and P035 target the X chromosome the gender of the reference samples used in an experiment is not important. Target probes are first normalised to reference probes within a sample.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the list of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number Female	Copy Number Male	Description
0.80 – 1.20	2	1	**Normal**

Typical Results of X Probes (*DMD*)

Final Ratio (FR)	Copy Number Female	Copy Number Male	Description
0	0	0	**Female:** Homozygous deletion **Male**: Deletion
0.40 – 0.65	1	-	**Female:** Heterozygous deletion
0.80 – 1.20	**2**	**1**	**Normal**
1.30 – 1.65	3	-	**Female:** Heterozygous duplication
1.75 – 2.15	4	-	**Female**: Homozygous duplication or Heterozygous triplication
1.65-2.25	-	2	**Male**: Duplication
All other values	-	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy numbers in normal and affected populations</td><td>To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the section above can be used. Cut-off values for copy number determination were verified with P034 DMD Mix 1 in 39 samples from healthy individuals, with normal copy numbers and four samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested with exception of the sample with a heterozygous partial DMD duplication affecting exons 2-30, for which three out of 38 measurements fell slightly below the cut-off range.

Cut-off values for copy number determination were verified with P035 DMD Mix 2 in 40 samples from healthy individuals, with normal copy numbers and four samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.</td></tr>
<tr><td>Limit of detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommended input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P034 DMD Mix 1 and P035 DMD Mix 2 on three samples with known CNV status and on two samples without any mutation. The sample with a heterozygous DMD duplication affecting exons 2-30 showed minor deviations to the expected FR values with both lower and upper input DNA with the use of P034 DMD Mix 1, but not P035 DMD Mix 2, for which the expected FRs were obtained. However, as only a few probes had ambiguous FR values, this would not lead to a false result, since the aberration in this sample is detected by 19 probes targeting DMD in the P034 DMD Mix 1 and all probe results are interpreted collectively. Furthermore, all other samples produced the expected results at both the lower and upper DNA input amounts.</td></tr>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using P034 DMD Mix 1 and P035 DMD Mix 2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNV status. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>P034: Expected FR for 611/615 measurements
P035: Expected FR for 629/630 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>P034: Expected FR for 615/615 measurements
P035: Expected FR for 628/630 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>P034: Expected FR for 615/615 measurements
P035: Expected FR for 630/630 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>P034: Expected FR for 615/615 measurements
P035: Expected FR for 629/630 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>P034: Expected FR for 611/615 measurements
P035: Expected FR for 605/630 measurements</td></tr>
</table>
* Results are summarised for all probes across all five samples tested.

An effect on the final ratios (FRs) was observed for a low number of probes with EDTA and Hemoglobin for both P034 DMD Mix 1 and P035 DMD Mix 2. NaCl and Heparin also affected a low number of P035 DMD Mix 2 probes. Hemoglobin had the largest effect on the FRs. Coffalyser.Net issued warnings for the samples in which Hemoglobin showed an effect, as well as lowered quality scores, leading to the samples needing a re-test.

To minimise variability across 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.</td></tr>
<tr><td>Cross-reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, three previously genotyped samples were tested using P034 DMD Mix 1 and P035 DMD Mix 2 and found to have the</td></tr>
</table>

	expected results, with the exception of a three deviating measurements with the use of P034 DMD Mix 1. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 3068/3075 data points for P034 DMD Mix 1, and in 2999/3000 data points for P035 DMD Mix 2, leading to a precision of >99% for both probemixes.
Clinical validity*	Approximately 60-70% of mutations in patients with DMD are deletions, 5-15% are duplications, and 20% are point mutations, small deletions or insertions in the *DMD* gene. In patients with BMD 60-70% of mutations are deletions, 20% are duplications, and 5-10% are point mutations, small deletions or insertions in the *DMD* gene (Duan et al. 2021). *(Based on a 2000-2025 literature review)

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

P034 Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
Xp21.2	*DMD*	Exon 70	1.8 kb	493	01392-L27986	
Xp21.2	*DMD*	Exon 69	2.4 kb	454	19341-L25594	+
Xp21.2	*DMD*	Exon 68	21.1 kb	413	02482-L28035	
Xp21.2	*DMD*	Exon 67	2.6 kb	386	01960-L33218	¥
Xp21.2	*DMD*	Exon 66	2.9 kb	341	01376-L25403	
Xp21.2	*DMD*	Exon 65	13.6 kb	312	19002-L26038	
Xp21.2	*DMD*	Exon 64	37.9 kb	268	01368-L25398	
Xp21.2	*DMD*	Exon 63	62.6 kb	241	01364-L25393	
Xp21.2	*DMD*	Exon 62	25.0 kb	199	01897-L25390	
Xp21.2	*DMD*	Exon 61	471.4 kb	165	01356-L25387	
Xp21.1	*DMD*	Exon 50	16.9 kb	476	19340-L25774	
Xp21.1	*DMD*	Exon 49	38.4 kb	436	19006-L24810	
Xp21.1	*DMD*	Exon 48	54.5 kb	400	23410-L33147	*
Xp21.1	*DMD*	Exon 47	2.4 kb	364	01378-L25405	
Xp21.1	*DMD*	Exon 46	36.3 kb	325	01374-L25401	
Xp21.1	*DMD*	Exon 45	248.6 kb	291	01370-L01287	
Xp21.1	*DMD*	Exon 44	70.6 kb	254	01366-L25396	
Xp21.1	*DMD*	Exon 43	22.6 kb	220	18221-L25392	
Xp21.1	*DMD*	Exon 42	32.0 kb	187	01711-L25389	
Xp21.1	*DMD*	Exon 41	69.6 kb	149	18831-L25385	
Xp21.1	*DMD*	Exon 30	26.5 kb	483	19008-L24812	
Xp21.1	*DMD*	Exon 29	3.0 kb	445	01387-L25413	
Xp21.1	*DMD*	Exon 28	7.3 kb	405	01716-L25410	
Xp21.1	*DMD*	Exon 27	6.2 kb	373	01379-L25406	
Xp21.1	*DMD*	Exon 26	8.8 kb	332	01375-L25402	
Xp21.1	*DMD*	Exon 25	1.2 kb	304	01371-L33217	¥
Xp21.1	*DMD*	Exon 24	3.8 kb	262	23408-L33145	*
Xp21.1	*DMD*	Exon 23	3.7 kb	226	19138-L25106	
Xp21.1	*DMD*	Exon 22	12.8 kb	193	18998-L24802	
Xp21.1	*DMD*	Exon 21	160.1 kb	157	01355-L25386	
Xp21.1	*DMD*	Exon 10	52.8 kb	469	01718-L26101	
Xp21.1	*DMD*	Exon 9	1.2 kb	427	01385-L25412	
Xp21.1	*DMD*	Exon 8	110.4 kb	393	23406-L33219	*
Xp21.1	*DMD*	Exon 7	7.0 kb	357	01713-L25404	
Xp21.1	*DMD*	Exon 6	6.7 kb	319	01373-L25725	
Xp21.1	*DMD*	Exon 5	21.5 kb	284	01954-L25724	
Xp21.1	*DMD*	Exon 4	5.0 kb	247	01365-L25394	
Xp21.1	*DMD*	Exon 3	170.4 kb	213	01361-L25391	
Xp21.1	*DMD*	Exon 2	191.2 kb	172	01357-L25388	
Xp21.1	*DMD*	Exon 1	0.2 kb	296	23405-L33216	*
Xp21.1	*DMD*	Exon 1		142	23404-L33100	*
Xp	Reference			462	23418-L26039	¥
Xp	Reference			231	00821-L09487	
Xp	Reference			130	13499-L02104	
Xp	Reference			380	16696-L33220	*
Xp	Reference			178	07655-L07361	
Xq	Reference			346	03149-L14468	
Xq	Reference			278	05893-L08952	
Xq	Reference			500	10764-L25900	
Xq	Reference			420	00820-L25090	
Xq	Reference			207	23417-L33215	¥

P035 Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
Xp21.2	*DMD*	Exon 79	6.2 kb	453	19142-L25437	
Xp21.2	*DMD*	Exon 78	7.6 kb	413	19342-L25595	+
Xp21.2	*DMD*	Exon 77	12.2 kb	381	01421-L25432	
Xp21.2	*DMD*	Exon 76	1.0 kb	342	01417-L25428	
Xp21.2	*DMD*	Exon 75	22.1 kb	310	01413-L25425	
Xp21.2	*DMD*	Exon 74	2.9 kb	269	01902-L25423	
Xp21.2	*DMD*	Exon 73	1.2 kb	239	01893-L25419	
Xp21.2	*DMD*	Exon 72	4.4 kb	199	01949-L25418	
Xp21.2	*DMD*	Exon 71	266.6 kb	165	23413-L33101	*
Xp21.2	*DMD*	Exon 60	33.6 kb	476	01431-L01977	¥
Xp21.2	*DMD*	Exon 59	0.8 kb	436	01427-L25436	
Xp21.2	*DMD*	Exon 58	17.8 kb	398	23412-L33103	*
Xp21.2	*DMD*	Exon 57	10.5 kb	364	01419-L25430	
Xp21.1	*DMD*	Exon 56	120.4 kb	326	23411-L33148	*
Xp21.1	*DMD*	Exon 55	30.3 kb	291	01411-L01058	
Xp21.1	*DMD*	Exon 54	21.3 kb	254	01894-L25421	
Xp21.1	*DMD*	Exon 53	50.2 kb	219	01892-L01050	
Xp21.1	*DMD*	Exon 52	44.4 kb	187	02059-L25417	
Xp21.1	*DMD*	Exon 51	569.2 kb	148	01394-L25415	
Xp21.1	*DMD*	Exon 40	2.8 kb	484	23409-L33232	*
Xp21.1	*DMD*	Exon 39	2.4 kb	445	19007-L24811	
Xp21.1	*DMD*	Exon 38	14.4 kb	407	19372-L25772	
Xp21.1	*DMD*	Exon 37	1.8 kb	372	03038-L26168	
Xp21.1	*DMD*	Exon 36	0.5 kb	332	01416-L25427	
Xp21.1	*DMD*	Exon 35	15.5 kb	303	15720-L25424	
Xp21.1	*DMD*	Exon 34	5.7 kb	263	01901-L25422	
Xp21.1	*DMD*	Exon 33	3.2 kb	226	19140-L25108	
Xp21.1	*DMD*	Exon 32	0.6 kb	193	18999-L24803	
Xp21.1	*DMD*	Exon 31	101.4 kb	157	01395-L25416	
Xp21.1	*DMD*	Exon 20	10.4 kb	470	01430-L33199	¥
Xp21.1	*DMD*	Exon 19	16.2 kb	427	19005-L24809	
Xp21.1	*DMD*	Exon 18	27.2 kb	388	01891-L25433	
Xp21.1	*DMD*	Exon 17	20.6 kb	358	01952-L25429	
Xp21.1	*DMD*	Exon 16	7.7 kb	319	02060-L25426	
Xp21.1	*DMD*	Exon 15	0.3 kb	283	19141-L25730	
Xp21.1	*DMD*	Exon 14	21.9 kb	247	19338-L25591	
Xp21.1	*DMD*	Exon 13	18.6 kb	211	19001-L24805	
Xp21.1	*DMD*	Exon 12	29.9 kb	172	23407-L33144	*
Xp21.1	*DMD*	Exon 11	695.1 kb	141	01393-L25414	
Xp21.1	*DMD*	Exon 1 Dp427c		490	01433-L33233	¥
Xp	Reference			232	07669-L07375	
Xp	Reference			297	06476-L26037	
Xp	Reference			130	13498-L06679	
Xp	Reference			463	13210-L14531	*
Xq	Reference			422	06187-L26204	
Xq	Reference			177	03110-L22383	
Xq	Reference			500	10764-L25900	
Xq	Reference			350	08245-L09531	
Xq	Reference			276	02900-L26167	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *DMD* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe changes

*	New probes.
¥	Probes changed in this product version. Minor alteration, no change in sequence detected.

Probe warnings

+ The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.

Probemix-specific precautions

1. This product 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).

2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Large deletions are often detected with this probemix. The slope correction algorithm in Coffalyser.Net may confuse a large *DMD* deletion and/or a deletion of a sequence targeted by a reference probe for sloping, leading to an incorrectly applied slope correction and a false warning or ambiguous results for multiple probes. Contact MRC Holland if you suspect that this happened.
6. Due to the presence of large introns surrounding certain exons in the *DMD* gene, consecutive probes may be located far apart in the genomic sequence. As a result, small deletions or duplications cannot always be verified by analysis of adjacent probes.
7. Dp427c is included in P035 DMD Mix 2 to provide complete coverage of the *DMD* gene. However, no clinical phenotype or interpretation has been reported for aberrations affecting Dp427c in the literature.
8. Prediction of the expected clinical phenotype based solely on the identified *DMD* variant should be made with caution. Factors such as whether the mutation is in-frame or out-of-frame, as well as the extent and location of the rearrangement, can significantly influence disease severity. Additionally, alternative promoter usage and transcript variability may modify the clinical outcome. The http://www.dmd.nl/ website has a tool that can assist in predicting the potential effect of exon deletions or duplications on the reading frame. However, confirmation at the RNA level is recommended, as DNA-based analyses do not always provide absolute certainty regarding the functional impact.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. For use on (un)cultured amniocytes, contamination of the sample with maternal DNA may lead to wrong conclusions.
2. For use on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and foetus have been described due to maternal contamination, postzygotic nondisjunction, postzygotic isochromosome formation, mosaic situations, and complications in DNA sampling in twin pregnancies (Van den Berg et al. 2006).

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Duan D et al. (2021). Duchenne muscular dystrophy. *Nature Reviews Disease Primers*. 7:13.
2. Van den Berg C et al. (2006). (Potential) false-negative diagnoses in chorionic villi and a review of the literature. *Prenat diagnosis*. 26:401-408.

Implemented changes in the product description

Version C1-01 – 09 March 2026 (03S)
- Product description adapted to a new template version.
- Intended purpose updated, specifying assay is manual and implementing name change of both probemixes to SALSA MLPA Probemixes P034 DMD Mix 1 and P035 DMD Mix 2.
- Warning for a ligation site >20nt from the nearest exon were added for probes 19341-L25594 and 19342-L25595.
- Several probes marked as new or changed in this product version.
- Probemixes are now IVDR-certified.

10. Appendix

P034 and P035 Combined Content – *DMD* Probes Sorted by Chromosomal Position

Length (nt) P034	Length (nt) P035	*DMD* Exon	Probe number	Distance to next probe
	490 ¥	Exon 1 Dp427c	01433-L33233	12.8 kb
142 *		Exon 1 Dp427m	23404-L33100	0.2 kb
296 *		Exon 1 Dp427m	23405-L33216	191.2 kb
172		Exon 2	01357-L25388	170.4 kb
213		Exon 3	01361-L25391	5.0 kb
247		Exon 4	01365-L25394	21.5 kb
284		Exon 5	01954-L25724	6.7 kb
319		Exon 6	01373-L25725	7.0 kb
357		Exon 7	01713-L25404	110.4 kb
393 *		Exon 8	23406-L33219	1.2 kb
427		Exon 9	01385-L25412	52.8 kb
469		Exon 10	01718-L26101	0.8 kb
	141	Exon 11	01393-L25414	29.9 kb
	172 *	Exon 12	23407-L33144	18.6 kb
	211	Exon 13	19001-L24805	21.9 kb
	247	Exon 14	19338-L25591	0.3 kb
	283	Exon 15	19141-L25730	7.7 kb
	319	Exon 16	02060-L25426	20.6 kb
	358	Exon 17	01952-L25429	27.2 kb
	388	Exon 18	01891-L25433	16.2 kb
	427	Exon 19	19005-L24809	10.4 kb
	470 ¥	Exon 20	01430-L33199	6.4 kb
157		Exon 21	01355-L25386	12.8 kb
193		Exon 22	18998-L24802	3.7 kb
226		Exon 23	19138-L25106	3.8 kb
262 *		Exon 24	23408-L33145	1.2 kb
304 ¥		Exon 25	01371-L33217	8.8 kb
332		Exon 26	01375-L25402	6.2 kb
373		Exon 27	01379-L25406	7.3 kb
405		Exon 28	01716-L25410	3.0 kb
445		Exon 29	01387-L25413	26.5 kb
483		Exon 30	19008-L24812	21.7 kb
	157	Exon 31	01395-L25416	0.6 kb
	193	Exon 32	18999-L24803	3.2 kb
	226	Exon 33	19140-L25108	5.7 kb
	263	Exon 34	01901-L25422	15.5 kb
	303	Exon 35	15720-L25424	0.5 kb
	332	Exon 36	01416-L25427	1.8 kb
	372	Exon 37	03038-L26168	14.4 kb
	407	Exon 38	19372-L25772	2.4 kb
	445	Exon 39	19007-L24811	2.8 kb
	484 *	Exon 40	23409-L33232	1.0 kb
149		Exon 41	18831-L25385	32.0 kb
187		Exon 42	01711-L25389	22.6 kb
220		Exon 43	18221-L25392	70.6 kb
254		Exon 44	01366-L25396	248.6 kb
291		Exon 45	01370-L01287	36.3 kb
325		Exon 46	01374-L25401	2.4 kb
364		Exon 47	01378-L25405	54.5 kb
400 *		Exon 48	23410-L33147	38.4 kb
436		Exon 49	19006-L24810	16.9 kb
476		Exon 50	19340-L25774	45.9 kb
	148	Exon 51	01394-L25415	44.4 kb
	187	Exon 52	02059-L25417	50.2 kb
	219	Exon 53	01892-L01050	21.3 kb
	254	Exon 54	01894-L25421	30.3 kb
	291	Exon 55	01411-L01058	120.4 kb
	326 *	Exon 56	23411-L33148	10.5 kb
	364	Exon 57	01419-L25430	17.8 kb
	398 *	Exon 58	23412-L33103	0.8 kb
	436	Exon 59	01427-L25436	33.6 kb
	476 ¥	Exon 60	01431-L01977	96.0 kb
165		Exon 61	01356-L25387	25.0 kb
199		Exon 62	01897-L25390	62.6 kb
241		Exon 63	01364-L25393	37.9 kb
268		Exon 64	01368-L25398	13.6 kb

312		Exon 65	19002-L26038	2.9 kb
341		Exon 66	01376-L25403	2.6 kb
386 ¥		Exon 67	01960-L33218	21.1 kb
413		Exon 68	02482-L28035	2.4 kb
454 +		Exon 69	19341-L25594	1.8 kb
493		Exon 70	01392-L27986	0.7 kb
	165 *	Exon 71	23413-L33101	4.4 kb
	199	Exon 72	01949-L25418	1.2 kb
	239	Exon 73	01893-L25419	2.9 kb
	269	Exon 74	01902-L25423	22.1 kb
	310	Exon 75	01413-L25425	1.0 kb
	342	Exon 76	01417-L25428	12.2 kb
	381	Exon 77	01421-L25432	7.6 kb
	413 +	Exon 78	19342-L25595	6.2 kb
	453	Exon 79	19142-L25437	