# Product Description
# SALSA® MLPA® Probemix P255-B1 ALDOB-FBP1

To be used with the MLPA General Protocol.

**Version B1**
No Changes.

**Catalogue numbers:**
- **P255-025R:** SALSA MLPA Probemix P255 ALDOB-FBP1, 25 reactions.
- **P255-050R:** SALSA MLPA Probemix P255 ALDOB-FBP1, 50 reactions.
- **P255-100R:** SALSA MLPA Probemix P255 ALDOB-FBP1, 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 P255-B1 ALDOB-FBP1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *ALDOB* and *FBP1*, which are associated with Hereditary fructose intolerance.

Hereditary fructose intolerance (HFI) is a rare recessive inherited disorder of carbohydrate metabolism, caused by catalytic deficiency of the aldolase B enzyme (ALDOB). The ALDOB enzyme plays a key role in glycolysis and gluconeogenesis and, in mammals, is preferentially expressed in the liver. HFI patients manifest hypoglycemia, lactic acidosis, and gastrointestinal symptoms, such as severe abdominal pain and recurrent vomiting after consuming fructose-containing foods. HFI usually presents in infancy at the time of weaning, when fructose is added to the diet. Persistent ingestion of fructose and related sugars (such as sucrose and sorbitol) can lead to severe liver and kidney damage, seizures, coma, and risk of death.

Mutations in the aldolase B gene (*ALDOB*) cause hereditary fructose intolerance (HFI). The protein expressed by this gene catalyses the cleavage of fructose-1-phosphate to form dihydroxyacetone phosphate and D-glyceraldehyde.

Hereditary fructose-1,6-diphosphatase deficiency is another form of fructose intolerance and exhibits similar signs and symptoms to HFI. This condition is inherited in an autosomal recessive way and caused by a deficiency in fructose-1,6-bisphosphatase 1 (FBP1), an enzyme which catalyses the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate and inorganic phosphate.

The *ALDOB* gene (9 exons), spans ~15.2 kb of genomic DNA and is located on chromosome 9q31.1, 101.4 Mb from p-telomere. The *FBP1* gene (8 exons), spans ~37.1 kb of genomic DNA and is located on chromosome 9q22.32, 96.4 Mb from p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK333439/.
More information is available at https://www.ncbi.nlm.nih.gov/books/NBK550349/.

**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 *ALDOB* exon numbering used in this P255-B1 ALDOB-FBP1 product description is the exon numbering from the RefSeq transcript NM_000035.4 (NG_012387.1), which is identical to the LRG_1244 sequence. The *FBP1* exon numbering is the exon numbering from the RefSeq transcript NM_001127628.2 (NG_008174.1). 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 or NG 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 P255-B1 contains 32 MLPA probes with amplification products between 163 and 445 nucleotides (nt). P255-B1 contains probes for each exon of the *ALDOB* gene, including two mutation-specific probes (A149P / c.448G>C; A174D / c.524C>A). In addition, one flanking probe targeting the *TGFBR1* gene located 2.3 Mb downstream of *ALDOB* is included. Furthermore, this probemix contains probes for each exon of the *FBP1* gene. In addition, twelve 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 Hereditary fructose intolerance. 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 SD030**

The SD030 Binning DNA provided with this probemix can be used for binning of all probes including the two mutation specific probes (*ALDOB* / exon 5 probe 08669-L08680, mutation c.448G>C and *ALDOB* / exon 5 probe 08670-L08682, mutation c.524C>A. SD030 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD030 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. 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). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD030 Binning DNA 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 *ALDOB and FBP1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P255-B1 ALDOB-FBP1 next to the A149P / c.448G>C; A174D / c.524C>A mutations.
- 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.

***ALDOB* and *FBP1* mutation databases**

https://databases.lovd.nl/shared/genes/ALDOB and https://databases.lovd.nl/shared/genes/FBP1. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 *ALDOB* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P255-B1 ALDOB-FBP1

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | *ALDOB* | *FBP1* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 163 | Reference probe 07904-L07642 | 5q | | |
| 172 | Reference probe 02978-L17087 | 4q | | |
| 178 | **FBP1** probe 20080-L27259 | | | **Exon 5** |
| 184 | **FBP1** probe 20081-L27260 | | | **Exon 8** |
| 193 | **ALDOB** probe 20131-L27365 | | **Exon 6** | |
| 202 | Reference probe 15424-L17583 | 7p | | |
| 210 | **FBP1** probe 20082-L27261 | | | **Exon 2** |
| 220 | Reference probe 02452-L01896 | 15q | | |
| 229 | **FBP1** probe 20083-L27262 | | | **Exon 4** |
| 238 | **FBP1** probe 20084-L27263 | | | **Exon 7** |
| 250 | **ALDOB** probe 20132-L27366 | | **Exon 4** | |
| 256 | **FBP1** probe 20085-L27264 | | | **Exon 6** |
| 265 ¬ | **TGFBR1** probe 04650-L04034 | | **Downstream** | |
| 274 | Reference probe 17450-L21206 | 16p | | |
| 284 | **ALDOB** probe 08666-L27273 | | **Exon 3** | |
| 292 | **ALDOB** probe 08672-L08684 | | **Exon 7** | |
| 300 | Reference probe 06366-L21564 | 18p | | |
| 311 | **FBP1** probe 20086-L27265 | | | **Exon 1** |
| 319 | **ALDOB** probe 08664-L25044 | | **Exon 1** | |
| 328 § | **ALDOB** probe 08669-L08680 | | **c.448G>C** | |
| 342 | Reference probe 12785-L25284 | 2q | | |
| 355 § | **ALDOB** probe 08670-L08682 | | **c.524C>A** | |
| 364 | **ALDOB** probe 08674-L27274 | | **Exon 9** | |
| 371 | **FBP1** probe 20087-L27597 | | | **Exon 3** |
| 382 | **ALDOB** probe 08668-L08678 | | **Exon 5** | |
| 392 | Reference probe 13587-L15044 | 1q | | |
| 401 | Reference probe 10638-L12897 | 8q | | |
| 409 | Reference probe 07455-L07103 | 17q | | |
| 418 | **ALDOB** probe 20133-L27367 | | **Exon 2** | |
| 427 | **ALDOB** probe 08673-L08685 | | **Exon 8** | |
| 436 | Reference probe 10731-L11313 | 6p | | |
| 445 | Reference probe 15086-L16849 | 12q | | |

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

§ Mutation-specific probe. This probe will only generate a signal when the **c.448G>C and c.524C>A** mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

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. P255-B1 probes arranged according to chromosomal location

Table 2a. *ALDOB* gene

| Length (nt) | SALSA MLPA probe | *ALDOB* exon[a] | Ligation site NM_000035.4 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *Start Codon* | *77-79 (exon 2)* | | |
| 319 | 08664-L25044 | Exon 1 | 8 nt before exon 1 | GTGTGCTACCAA-AGATCTGTCTTA | 5.0 kb |
| 418 | 20133-L27367 | Exon 2 | 186-187 | AGATGAATCTGT-AGGTGAGTGTAA | 1.0 kb |
| 284 | 08666-L27273 | Exon 3 | 376-377 | ATCCTCAAGGAA-AAGGGGATCGTG | 1.3 kb |
| 250 | 20132-L27366 | Exon 4 | 448-449 | AAAGAAACCACC-ATTCAAGGTAAG | 0.8 kb |
| 382 | 08668-L08678 | Exon 5 | 461-462 | TTGCAGGGCTTG-ATGGCCTCTCAG | 0.1 kb |
| 328 § | 08669-L08680 | Exon 5 | 524-525 | GGAAGTGGCGTC-CTGTGCTGAGGA | 0.1 kb |
| 355 § | 08670-L08682 | Exon 5 | 600-601 | GGCTCGCTACGA-CAGCATCTGTCA | 1.0 kb |
| 193 | 20131-L27365 | Exon 6 | 4 nt after exon 6 | CTGAGAAGGTAA-GTTTTAAATATG | 1.1 kb |
| 292 | 08672-L08684 | Exon 7 | 858-859 | CCACCGTACTGT-TCCTGCAGCTGT | 0.5 kb |
| 427 | 08673-L08685 | Exon 8 | 973-974 | TGGAAACTAAGT-TTCTCTTATGGA | 3.1 kb |
| 364 | 08674-L27274 | Exon 9 | 1119-1120 | TCACACGGGTTC-TTCTGGGGCTGC | 2279.3 kb |
| | | *Stop Codon* | *1169-1171 (exon 9)* | | |
| | | | | | |
| 265 ¬ | 04650-L04034 | *TGFBR1* | | TTGGTGTCAGAT-TATCATGAGCAT | |

Table 2b. *FBP1* gene

| Length (nt) | SALSA MLPA probe | *FBP1* exon[a] | Ligation site NM_001127628.2 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *Start Codon* | *245-247 (exon 2)* | | |
| 311 | 20086-L27265 | Exon 1 | 182-183 | ATCTTGAGAAGA-GGCCTGGATTAA | 0.9 kb |
| 210 | 20082-L27261 | Exon 2 | 404-405 | GCAAGGCGGGCA-TCGCGCACCTGT | 18.8 kb |
| 371 | 20087-L27597 | Exon 3 | 496-497 | CTGGTTATGAAC-ATGTTAAAGTCA | 2.6 kb |
| 229 | 20083-L27262 | Exon 4 | 607-608 | TTTGATCCCCTT-GATGGATCTTCC | 7.9 kb |
| 178 | 20080-L27259 | Exon 5 | 787-788 | ATGGACTGTGGG-GTCAACTGCTTC | 3.1 kb |
| 256 | 20085-L27264 | Exon 6 | 868-869 | AAGAAAGGTAAA-ATCTACAGCCTT | 1.3 kb |
| 238 | 20084-L27263 | Exon 7 | 1 nt before exon 7 | TCTTAACTTGCA-GGATAATTCAGC | 2.1 kb |
| 184 | 20081-L27260 | Exon 8 | 1163-1164 | TGTTAGACGTCA-TTCCCACAGACA | |
| | | *Stop Codon* | *1259-1261 (exon 8)* | | |

[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.

§ Mutation-specific probe. This probe will only generate a signal when the **c.448G>C and c.524C>A** mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

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

- P117 ABCC8: Contains probes for the *ABCC8* gene, involved in familial hyperinsulinemic hypoglycaemia.
- P156 GALT: Contains probes for the *GALT* gene, involved in classic galactosaemia.

## 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.


## Selected publications using SALSA MLPA Probemix P255 ALDOB-FBP1


- Santer R et al. (2016). A summary of molecular genetic findings in fructose-1,6-bisphosphatase deficiency with a focus on a common long-range deletion and the role of MLPA analysis. *Orphanet Journal of Rare Diseases*. 21;11:44.
- Ferri L et al. (2012). Integration of PCR-Sequencing Analysis with Multiplex Ligation-Dependent Probe Amplification for Diagnosis of Hereditary Fructose Intolerance. *JIMD Report* 6:31-7. doi: 10.1007/8904_2012_125.


| **P255 product history** | |
|---|---|
| *Version* | *Modification* |
| B1 | Probes for new gene *FBP1* added, several target probes and reference probes have been replaced/added. |
| A2 | The 88, 96, 100 and 105 nt control fragments (QDX2) have been included. |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version B1-01– 14 January 2021 (04P)<br>- Product description rewritten and adapted to a new template.<br>- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).<br>- Ligation sites of the probes targeting the *ALDOB* and *FBP1* genes updated according to new versions of the NM_ reference sequence.<br>*Version 09 — 02 June 2017 (55)*<br>- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).<br>- New references added on page 2.<br>- Minor textual changes throughout the document. |


| **More information: www.mrcholland.com; www.mrcholland.eu** | |
|---|---|
| | MRC Holland bv; Willem Schoutenstraat 1<br>1057 DL, Amsterdam, The Netherlands |
| E-mail | info@mrcholland.com (information & technical questions)<br>order@mrcholland.com (orders) |
| Phone | +31 888 657 200 |