Product Description SALSA® MLPA® Probemix P283-B2 TPMT

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, two reference probes have been removed and three reference probes have been replaced. For complete product history see page 6.

Catalogue numbers:
- **P283-025R:** SALSA MLPA Probemix P283 TPMT, 25 reactions.
- **P283-050R:** SALSA MLPA Probemix P283 TPMT, 50 reactions.
- **P283-100R:** SALSA MLPA Probemix P283 TPMT, 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.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 P283 TPMT is a **research use only (RUO)** assay for the detection of deletions or duplications in the *TPMT* gene, which is associated with thiopurine S-methyltransferase deficiency (TPMT deficiency). This probemix can also be used to detect the presence of the c.460A>G/p.A154T (TPMT*3B allele), c.719A>G/p.Y240C (TPMT*3C allele), or both (TPMT*3A allele), and c.238G>C/p.A80P (TPMT*2 allele) point mutations.

Defects in the thiopurine S-methyltransferase (*TPMT*) gene are the cause of TPMT deficiency. TPMT is an enzyme involved in the normal metabolic inactivation of thiopurine drugs. These drugs are generally used as immunosuppressant or cytotoxic drugs and are prescribed for a variety of clinical conditions including leukaemia, autoimmune disease, and organ transplantation. Patients with intermediate or no TPMT activity are at risk of toxicity after receiving standard doses of thiopurine drugs and it is shown that inter-individual differences in response to these drugs are largely determined by genetic variation at the *TPMT* locus.

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 *TPMT* exon numbering used in this P283-B2 TPMT product description is the exon numbering from the RefSeq transcript NM_000367.4, which is identical to the LRG_874 sequence. The exon numbering and NM_ sequence used have been retrieved on 12/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P283-B2 TPMT contains 22 MLPA probes with amplification products between 130 and 380 nucleotides (nt). This includes nine probes for the *TPMT* gene, one probe for each of the nine exons, with the exception of exon 6, and two probes for exon 2. In addition, this probemix contains probes specific for the A154T and A80P mutations, which will only generate a signal when the mutations are present, and one probe detecting the wildtype sequence of the Y240C mutation, a reduced signal can point towards the presence of this mutation **or** a deletion of exon 9. In addition, ten 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.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, 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 nt and 96 nt fragment 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.mlpa.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 unrelated individuals who are from families without a history of TPMT deficiency. 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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.

SALSA Binning DNA SD087: The SD087 Binning DNA provided with this probemix can be used for binning of two mutation-specific probes (314 nt probe 17379-SP0499-L22511 A154T mutation and 277 nt probe 17378-SP0500-L22512 A80P mutation). SD087 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD087 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 signals, as for this purpose true mutation positive patient samples or cell lines should be used. 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 SD087 Binning DNA product description, available online: www.mlpa.com.

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 each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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 (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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *TPMT* gene are small (point) mutations, next to the before mentioned mutations, most of which will not be detected by using SALSA MLPA Probemix P283 TPMT.
- 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.

***TPMT* mutation database:** https://databases.lovd.nl/shared/genes/TPMT. 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 SNPs and unusual results (e.g., a duplication of *TPMT* exons 2 and 4 but not exon 3) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P283-B2 TPMT

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*TPMT*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
142	**TPMT probe** 09454-L09710		**Exon 8**
160	Reference probe 08901-L08992	11p11	
166	Reference probe 01642-L01219	18q21	
181	Reference probe 16394-L19456	17q22	
202	**TPMT probe** 17377-L21065		**Exon 3**
220	**TPMT probe** 09446-L09702		**Exon 1**
227	**TPMT probe** 09450-L21063		**Exon 4**
235 ∞ Ж	**TPMT probe** 09455-SP0048-L21064		Y240C
244 *	Reference probe 22141-L31497	16p13	
255	**TPMT probe** 09448-L09704		**Exon 2**
265	**TPMT probe** 09451-L09707		**Exon 5**
277 § Ж	**TPMT probe** 17378-SP0500-L22512		A80P
285	Reference probe 14687-L08626	12p12	
292	**TPMT probe** 09456-L09713		**Exon 9**
303	**TPMT probe** 09453-L09709		**Exon 7**
314 § Ж	**TPMT probe** 17379-SP0499-L22511		A154T
320	Reference probe 14346-L16015	2q32	
337 *	Reference probe 21529-L29738	7q31	
348 *	Reference probe 19887-L26752	10q25	
364	**TPMT probe** 09447-L09703		**Exon 2**
380	Reference probe 16932-L19875	4q12	

a) See above section on exon numbering for more information.

* New in version B2.
§ Mutation-specific probes. These probes will only generate a signal when the A154T and A80P mutations are present. It has been tested on artificial DNA **but not on positive human samples!**
∞ Wild type sequence detected. The presence of the Y240C mutation **or** a deletion of exon 9 will result in a decreased probe signal.
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.

Table 2. *TPMT* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*TPMT* exon[a]	Ligation site NM_000367.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*182-184 (Exon 2)*		
220	09446-L09702	Exon 1	125-126	GGCCGGCAACCA-GCTGTAAGCGAG	5.9 kb
364	09447-L09703	Exon 2	10 nt before exon 2	ATTGCAAATATT-TTCCACGTAGGC	0.2 kb
255	09448-L09704	Exon 2	313-314	CATCAGGAACAA-GGACATCAGTAA	1.2 kb
202	17377-L21065	Exon 3	405-406	AAAAGCGGTTGA-GATGAAATGGTA	4.1 kb
277 § Ж	17378-SP0500-L22512	A80P (Exon 4)	44 nt before exon 4; 419-420	CCTCTATTTAGT-49 nt spanning oligo-CAGACCGGGGAC	0.1 kb
227	09450-L21063	Exon 4	545-546	CCAAAGTATTTA-AGGTTTGTTTTG	3.9 kb
265	09451-L09707	Exon 5	2 nt after exon 5	ATCTTCCCAGGT-AGGTTGAATACT	0.7 kb
314 § Ж	17379-SP0499-L22511	A154T (Exon 6)	641-642; 14 nt after exon 6	GGGATAGAGGAA-48 nt spanning oligo-TTTTTTTGTTTA	5.0 kb
303	09453-L09709	Exon 7	30 nt before exon 7	GTGTAGAGAAAT-GTAACAAATACC	1.8 kb
142	09454-L09710	Exon 8	800-801	AAATTGAAAGGT-TGTTTGGTAAGT	1.5 kb
235 ∞ Ж	09455-SP0048-L21064	Y240C (Exon 9)	900-901; 943-944	TGAAAAGTTATA-43 nt spanning oligo-ACACTGACATGT	0.3 kb
292 #	09456-L09713	Exon 9	1204-1205	TACCAATCAGCA-TGTGTTACCTGT	
		stop codon	*917-919 (Exon 9)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
§ Mutation-specific probes. These probes will only generate a signal when the A154T and A80P mutations are present. It has been tested on artificial DNA **but not on positive human samples!**
∞ Wild type sequence detected. The presence of the Y240C mutation **or** a deletion of exon 9 will result in a decreased probe signal.
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.

Related SALSA MLPA probemixes

P103 DPYD Contains probes for the *DPYD* gene.

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.

P283 Product history	
Version	*Modification*
B2	Two reference probes have been removed and three reference probes have been replaced.
B1	Two additional mutation specific probes for *TPMT* (A80P; A154T) have been included. The *TPMT* exon 6 probe and the DPYD probes have been removed. All reference probes (except one) have been replaced and four additional reference probes have been included.
A1	First release.

Implemented changes in the product description
Version B2-01 — 16 January 2020 (02P) - 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 *TPMT* gene updated according to new version of the NM_ reference sequence. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. *Version 11 – 19 May 2016 (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. - "Peak area" replaced with "peak height". - Update link for "Database of Genomic Variants". - Manufacturer's address adjusted. *Version 10 – 03 August 2015 (49)* - Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed. *Version 09 (49)* - Warning added in Table 1 and 2, 227 nt probe 09450-L21063 and 255 nt probe 09448-L09704. *Version 08 (48)* - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). - Various minor textual changes. *Version 07 (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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