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 7.

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

SALSA® MLPA® Probemix P283 TPMT (hereafter: P283 TPMT) 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.)

P283 TPMT can be used in combination with:

3. SALSA® Binning DNA SD087 (Cat. No: SD087)

Volumes and ingredients

Volumes			Ingredients
P283-025R	**P283-050R**	**P283-100R**	
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 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 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.460G>A/p.A154T (TPMT*3B allele), c.719A>G/p.Y240C (TPMT*3C allele), or both (TPMT*3A allele), and the 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 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: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Locus Reference Genomic (LRG) database: https://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. 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
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 of this probe can point towards the presence of the 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.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 MLPA (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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from 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 (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 mutation-specific probes can only detect the presence of the mutation and should not be used to determine zygosity. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

SALSA® Binning DNA SD087
The SALSA® Binning DNA SD087 provided with this probemix can be used for binning of all probes including the two mutation-specific probes (314 nt probe 17379-SP0499-L22511 for the A154T mutation and 277 nt probe 17378-SP0500-L22512 for the A80P mutation). SD087 is a mixture of human female genomic DNA from healthy individuals and a titrated amount of synthetic DNA that contains the target sequence detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD087 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 signals. For further details, please consult the SD087 product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 (with exception of the mutation-specific probes) 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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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.

The above mentioned FR values do not apply to the mutation-specific probes. The peaks of the mutation-specific probes are expected to be absent in the majority of samples tested and therefore their standard deviation cannot be determined. Clear signal (at least 10% of the median peak height of all reference probes in that sample) for one of these probes indicates that the mutation is present.

- 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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: https://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 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 *TPMT* gene are small (point) mutations, none of which will be detected by using P283 TPMT. Three point mutations in the *TPMT* gene can be detected, but other point mutations in the *TPMT* gene cannot be detected.
- 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 as well as point mutations always require confirmation by another method. Because the mutation-specific probes are only intended to determine the presence of the mutation, positive results obtained for either of these probes need to be confirmed by sequence analysis to determine the zygosity of the mutation. 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 in sequence data 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 https://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 *TPMT* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. P283-B2 TPMT

Length (nt)	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 section Exon numbering on page 2 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. A lowered probe signal can be due to a Y240C mutation **or** a deletion of exon 9. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b] NM_000367.4	Partial sequence[c] (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 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.

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.
§ 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. A lowered probe signal can be due to a Y240C mutation **or** a deletion of exon 9. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
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.

SNVs located in the target sequence of a probe can influence probe hybridisation 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.

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 B2-02 – 16 May 2025 (05P) - Product description adapted to a new template. - Various minor textual changes. *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.

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