Product Description
SALSA® MLPA® Probemix P383-A2 T-ALL

To be used with the MLPA General Protocol.

Version A2
For complete product history see page 11.

Catalogue numbers:
- **P383-025R:** SALSA MLPA Probemix P383 T-ALL, 25 reactions.
- **P383-050R:** SALSA MLPA Probemix P383 T-ALL, 50 reactions.
- **P383-100R:** SALSA MLPA Probemix P383 T-ALL, 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 P383 T-ALL is a **research use only (RUO)** assay for the detection of deletions or duplications in 13 different chromosomal regions, which are suggested to be of diagnostic and/or prognostic importance in T-ALL.

T-lineage acute lymphoblastic leukemia (T-ALL) is a clonal malignant disorder of immature T-cells, which accounts for about 15% of paediatric and 25% of adult ALL cases (Iacobucci and Mullighan 2017). Although prognosis has improved due to effective treatment protocols, around 25% of paediatric and 50-70% of adult patients still experience relapse. T-ALL is genetically heterogeneous, and comprises multiple distinct subtypes defined by chromosomal rearrangements that usually involve one of the T-cell receptor (TCR) loci, but non-TCR-driven translocations are also detected. In addition, genome-wide characterisation has identified several novel amplifications, deletions and point mutations in T-ALL samples that are of interest to better define T-ALL subgroups (Girardi et al. 2017).

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
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/
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
From product description version A2-05 onwards, the exon numbering from the MANE Select transcripts is used for all the target genes, unless otherwise specified, please see Table 2. Please be aware that the MANE and LRG exon numbering do not always correspond, and MANE exon numbering used here may differ from literature. 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. Please note that exon numbering for the same gene might be

different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MLPA Probemix P383-A2 T-ALL contains 56 MLPA probes with amplification products between 130 and 504 nucleotides (nt). This includes probes for 13 chromosomal regions, including *STIL-TAL1* (1p33), *LEF1* (4q25), *CASP8AP2* (6q15), *MYB* (6q23), *EZH2* (7q36) *MLLT3+MTAP+CDKN2A/B* (9p21), *NUP214-ABL1* (9q34), *PTEN* (10q23), *LMO1* (11p15), *LMO2* (11p13), *NF1+SUZ12* (17q11), *PTPN2* (18p11) and *PHF6* (Xq26). In this probemix, 40 out of the 56 MLPA probes are used as reference probes, as they are spread over a number of different chromosomal regions and it is expected that the majority of these probes will have a normal copy number in most samples. Complete/partial probe sequences are available in Table 2 and 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals of the same sex 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 reference 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 healthy individuals without a history of acute lymphoblastic leukemia. It is recommended to use samples of the same sex to facilitate interpretation. 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
See the section “Positive samples” on the P383 product page on our website.

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 reference probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication/gain		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subconal cases.
- 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.
- 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.

P383 specific notes

- In case data analysis is performed with a software other than Coffalyse.Net, we recommend to use all probes as reference probes with the exception of the chromosome 9 and Xq probes.
- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- In most populations, the major cause of genetic alterations in the genes included in this probemix are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P383 T-ALL.
- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in tumours with more chaotic karyotypes.

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.

COSMIC mutation database

http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC Database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report, false positive results due to SNVs and unusual results (e.g., a deletion of *LEF1* exons 1 and 6 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P383-A2 T-ALL

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		Used as reference probe	Location (hg18) in kb
		Chromosomal band	Exon		
64-105	Control fragments – see table in probemix content section for more information				
130	**STIL probe** 15947-L18099	1p33	Exon 1	Ref	01-047,552
137 ¥	**PHF6 probe** 18550-L29679	Xq26.2	Exon 1	-	X-133,335
142	**TAL1 probe** 13063-L23841	1p33	Intron 1	Ref	01-047,468
147	**MYB probe** 12500-L18025	6q23.3	Exon 2	Ref	06-135,549
152	**RAG2 probe** 18551-L23889	11p12	Exon 2	Ref	11-036,572
157	**MLLT3 probe** 12533-L23842	9p21.3	Exon 1	-	09-020,612
161	**PHF6 probe** 15948-L23843	Xq26.2	Exon 3	-	X-133,340
166	**CASP8AP2 probe** 15949-L23844	6q15	Exon 1	Ref	06-090,596
170	**MLLT3 probe** 16058-L23880	9p21.3	Exon 7	-	09-020,353
176 Ж ±	**PTPN2 probe** 15951-SP0316-L23881	18p11.21	Exon 1	Ref	18-012,874
182	**CDKN2A probe** 11869-L19013	9p21.3	Upstream	-	09-021,965
190 ¬	SH2D1A probe 06104-L05559	Xq25	-	-	X-123,308
196	**LMO2 probe** 12538-L23846	11p13	Exon 6	Ref	11-033,837
202 ¬	ARHGEF6 probe 02902-L02296	Xq26.3	-	-	X-135,585
208 Ж	**PTPN2 probe** 18549-SP0318-L23866	18p11.21	Exon 4	Ref	18-012,821
214	**PTEN probe** 13691-L23847	10q23.31	Exon 7	Ref	10-089,708
220	**LEF1 probe** 12507-L24045	4q25	Exon 1	Ref	04-109,309
226	**PHF6 probe** 15954-L23884	Xq26.2	Exon 7	-	X-133,376
233	**PTEN probe** 13685-L17760	10q23.31	Exon 1	Ref	10-089,614
239 Ж	**PTPN2 probe** 15955-SP0317-L18107	18p11.21	Exon 2	Ref	18-012,849
244 Ж	**LMO1 probe** 16714-SP0381-L23885	11p15.4	Exon 2	Ref	11-008,209
250	**NF1 probe** 03849-L18072	17q11.2	Exon 26	Ref	17-026,584
257	**CDKN2B probe** 17935-L23887	9p21.3	Exon 2	-	09-021,996
263	**EZH2 probe** 18269-L23383	7q36.1	Exon 3	Ref	07-148,175
269	**CASP8AP2 probe** 15957-L18109	6q15	Exon 2	Ref	06-090,613
274	**STIL probe** 15112-L16883	1p33	Exon 6	Ref	01-047,538
281 ¥	**CDKN2A probe** 16533-L29647	9p21.3	Exon 3	-	09-021,958
287	**ABL1 probe** 18552-L24043	9q34.12	Exon 3	-	09-132,720
292	**MYB probe** 17265-L20654	6q23.3	Exon 16	Ref	06-135,581
299 ¬	AHI1 probe 15960-L18112	6q23.3	-	Ref	06-135,648
304	**EZH2 probe** 18272-L23021	7q36.1	Exon 14	Ref	07-148,143
311	**SUZ12 probe** 18705-L23805	17q11.2	Exon 15	Ref	17-027,348
319 ¥	**ABL1 probe** 12516-L29680	9q34.12	Exon 11	-	09-132,749
326	**MYB probe** 00441-L24480	6q23.3	Exon 6	Ref	06-135,555
334	**MTAP probe** 15678-L22421	9p21.3	Exon 1	-	09-021,793
343 Ж	**LMO1 probe** 16717-SP0380-L19731	11p15.4	Exon 4	Ref	11-008,203
351	**RAG2 probe** 16520-L23853	11p12	Exon 1	Ref	11-036,576
361	**NUP214 probe** 18553-L23891	9q34.13	Exon 2	-	09-132,993
371 ¥	**TAL1 probe** 12520-L29681	1p33	Exon 5	Ref	01-047,458
377	**LEF1 probe** 15964-L23859	4q25	Exon 6	Ref	04-109,222
382	**LMO2 probe** 12558-L23858	11p13	Exon 1	Ref	11-033,870
391	**PHF6 probe** 15965-L24481	Xq26.2	Exon 11	-	X-133,388
400	**NF1 probe** 02530-L23856	17q11.2	Exon 58	Ref	17-026,725
409	**CASP8AP2 probe** 15967-L23854	6q15	Exon 6	Ref	06-090,624
417	**LEF1 probe** 15968-L18120	4q25	Exon 3	Ref	04-109,304
426	**CD44 probe** 00669-L23838	11p13	-	Ref	11-035,207
433	**CASP8AP2 probe** 15969-L24482	6q15	Exon 11	Ref	06-090,640
439	**PTEN probe** 13692-L23864	10q23.31	Exon 7	Ref	10-089,708
445	**STIL probe** 15125-L23865	1p33	Exon 12	Ref	01-047,519
454	**LEF1 probe** 12526-L29682	4q25	Exon 12	Ref	04-109,189
462	**PTPN2 probe** 15970-L23850	18p11.21	Exon 9	Ref	18-012,784

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		Used as reference probe	Location (hg18) in kb
		Chromosomal band	Exon		
469 Ж	**EZH2 probe** 18555-SP0635-L23020	7q36.1	Exon 20	Ref	07-148,135
475	**PTEN probe** 13696-L17520	10q23.31	Exon 9	Ref	10-089,715
481 ¥	**SLC1A2 probe** 18698-L29605	11p13	-	Ref	11-035,244
492	**NUP214 probe** 18547-L24044	9q34.13	Exon 23	-	09-133,041
504	**SUZ12 probe** 18539-L23848	17q11.2	Exon 10	Ref	17-027,340

[a] See section Exon numbering on page 1 for more information.

¥ Changed in version A2. Minor alteration, no change in sequence detected.

± SNP rs200164592 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or 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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P383-A2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location (hg18)/ Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
***STIL-TAL1* fusion**, at 1p33, indicated ligation sites and exon numbering for *TAL1* are according to the MANE Select transcript NM_001290403.2, and for *STIL* according to the MANE Select transcript NM_001048166.1. Sub-microscopic interstitial deletions (~90 kb, most commonly starting at chr1:047,470,111 and ending at chr1:047,552,032; hg18) encompassing the 5' UTR region of the *TAL1* gene and the last *STIL* exons, lead to a *STIL-TAL1* fusion gene resulting in increased expression of *TAL1* (Brown et al. 1990). A *STIL-TAL1* fusion is found in 10-30% of T-ALL cases with a normal karyotype and is suggested to be associated with a relatively favourable outcome (Cave et al. 2004). ^ Decreased signal of the probes for *STIL* exons 6 and 12 is indicative for *STIL-TAL1* fusion gene. NOTE: The exon numbering used in previous versions of this product description, can be found in between brackets.					
371	12520-L29681	***TAL1***, ex 5 (6)	824-825	GCAGCAGAATGT-GAACGGGGCCTT	9.4 kb
142	13063-L23841	***TAL1***, intr 1 (3)	120 nt before ex 2	CCCTGGACTGGT-TGGTCGGGGACA	51.5 kb
445 ^	15125-L23865	***STIL***, ex 12	1660-1661	TCTTTTGAGACA-CTGCAAAGTAAG	19.1 kb
274 ^	15112-L16883	***STIL***, ex 6	668-669	CTGCTTTCCCTA-AGAGTTCATATC	14.0 kb
130	15947-L18099	***STIL***, ex 1	26-27	CAACGTCCCAGA-GGCGGGGCCGGA	-
***LEF1* gene**, at 4q25, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_016269.5. Mono/biallelic microdeletions of *LEF1* are detected in 11% of children with T-ALL, and its inactivation seems to correlate with better survival and with younger age at diagnosis. The first exons of *LEF1* are frequently deleted (Gutierrez et al. 2010).					
454	12526-L29682	***LEF1***, ex 12	2539-2540	CTAGAGACGCTG-ATCCATAAAGAC	33.1 kb
377	15964-L23859	***LEF1***, ex 6	1831-1832	TATCCCATCACG-GGTGGATTCAGG	82.0 kb
417	15968-L18120	***LEF1***, ex 3	1533-1534	TATGAATAACGA-CCCATACATGTC	4.6 kb
220	12507-L24045	***LEF1***, ex 1	697-698	GCATCCGGGGTA-ACTACAGTGGAG	-
***CASP8AP2* gene**, at 6q15, indicated ligation sites and exon numbering are according to the transcript NM_012115.4. Deletion of 6p15-p16.1, including the *CASP8AP2* gene, is detected in 12% of T-ALL patients and is suggested to associate with poor early treatment response (Remke et al. 2009). NOTE: There is no MANE or LRG reference sequence for this gene, thus exon numbering for this gene is based on NM_012115.4 transcript.					
166	15949-L23844	***CASP8AP2***, ex 1	127-128	GTAGTCCCCCGA-GTGGAGGTCGGC	16.6 kb
269	15957-L18109	***CASP8AP2***, ex 2	255-256	GGAAATAGGATC-ATGGCAGCAGAT	10.5 kb
409	15967-L23854	***CASP8AP2***, ex 6	596-597	TCTTAAGAAGAA-TATTTCAGCACT	16.8 kb
433	15969-L24482	***CASP8AP2***, ex 11	6367-6368	TTTCACATCAGC-ATGTTCAGCTTG	45 **M**b

***MYB* gene**, at 6q23.3, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_001130173.2.
Duplication of the *MYB* oncogene is detected in 8-15% of the individuals with T-ALL (Clappier et al. 2007; O'Neil et al. 2007). In most cases, the 3' end of the *AHI1* gene is also duplicated together with *MYB*. The sub-microscopic size of the aberration makes this duplication undetectable by conventional cytogenetics/FISH in most cases (Lahortiga et al. 2007).

147	12500-L18025	***MYB***, ex 2	265-266	TGAGGACTTTGA-GATGTGTGACCA	6.4 kb
326	00441-L24480	***MYB***, ex 6	782-783	ATGCGTCGGAAG-GTCGAACAGGAA	26.1 kb
292	17265-L20654	***MYB***, ex 16	2969-2970	CATTTAATCCAG-ATTGTAAATGCT	66.9 kb
299 ¬	15960-L18112	*AHI1*	6q23.3	GAAAACACTGTA-ATTGCTACTCAG	-

***EZH2* gene**, at 7q36.1, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_004456.5.
The *EZH2* gene has been shown to be inactivated by loss-of-function mutations and deletions in up to 25% of T-ALL patients (Ntziachristos et al. 2012; Zhang et al. 2012). Moreover, loss of *EZH2* in hematopoietic stem cells has been shown to cause aggressive T-ALL in mice (Simon et al. 2012).

469 Ж	18555-SP0635-L23020	***EZH2***, ex 20	2605-2606 and 2649-2650	TTTTGCAATAAT-44nt spanning oligo-TTGTCCTTGTTG	7.6 kb
304	18272-L23021	***EZH2***, ex 14	1761-1762	TGCCCTTGTGTG-ATAGCACAAAAT	31.6 kb
263	18269-L23383	***EZH2***, ex 3	318-319	CAAGAATGGAAA-CAGCGAAGGATA	-

***CDKN2A/B*, *MLLT3* and *MTAP* genes,** at 9p21.3, indicated ligation sites and exon numbering for *MLLT3* are according to the MANE Select transcript NM_004529.4.
CDKN2A/B inactivation is found in 50-60% of all T-ALL cases. In a considerable number of patient samples, this deletion is sub-microscopic and is not detectable by conventional cytogenetics or by FISH (Usvasalo et al. 2008; Sulong et al. 2009). Another suggested target gene in the chromosomal region is *MLLT3*, which is deleted in 26% of T-ALL patients (Mullighan et al. 2007). *CDKN2A/B* deletions are suggested to associate with poor outcome in both paediatric and adult T-ALL (Fizzotti et al. 1995; Yamada et al. 1997).
From product description version A2-04 onwards, the exon numbering from the MANE transcripts is used for *CDKN2A*. Consequently, for *CDKN2A*, the exon numbering has been changed: NM_000077.5 (MANE Select) encoding p16INK4A and NM_058195.4 (MANE Plus Clinical) encoding p14ARF are used. Both NM_000077.5 and NM_058195.4 have distinct first exons (both numbered as exon 1) which contain the translation start codon, and share a common second exon, which is translated in different reading frames. The exon numbering used in previous versions of this product description can be found in between brackets. **Please be aware that the MANE and LRG exon numbering do not correspond for *CDKN2A*, and MANE exon numbering used here may differ from literature.**

170	16058-L23880	***MLLT3***, ex 7	1524-1525	GATAACGACAAT-GACTCTGAAATG	258.9 kb
157	12533-L23842	***MLLT3***, ex 1	124-125	TGTCCGCCATCT-ACCCTCCGCTCC	1.2 **M**b
334 #	15678-L22421	***MTAP***	9p21.3	GCCCACTGCAGA-TTCCTTTCCCGT	165.5 kb
281	16533-L29647	***CDKN2A***, ex 3 (4)	NM_000077.5 & NM_058195.4; 33 nt before ex 3	TTGACCTCAGGT-TTCTAACGCCTG	7.0 kb
182	11869-L19013	***CDKN2A*, upstream** (intron 1)	NM_000077.5; 378 nt before ex 1; NM_058195.4; 4.0 kb before ex 2	CACGCCTTTGCT-GGCAGGCGGGGG	30.6 kb
257	17935-L23887	***CDKN2B***	9p21.3	GCCTGTCTGAGA-CTCACAGGAAGG	111 **M**b

***NUP214-ABL1* fusion gene**, at 9q34.12-q34.13, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_005157.6 for *ABL1* and NM_005085.4 for *NUP214*.
Amplification of the *NUP214-ABL1* fusion gene is observed in 6% of T-ALL and is suggested to be associated with an aggressive disease course (Graux et al. 2004; Graux et al. 2009). Detection of *NUP214-ABL1* fusion amplification is potentially both of prognostic and clinical significance, as patients with *NUP214-ABL1* fusion amplification could benefit from treatment with tyrosine kinase inhibitors.
NOTE: The exon numbering (LRG_769 for *ABL1*), used in previous versions of this product description, can be found in between brackets.

287	18552-L24043	***ABL1*, ex 3 (4)**	508-509	GCCCAAACCAAA-AATGGCCAAGGC	29.2 kb
319	12516-L29680	***ABL1*, ex 11 (12)**	1923-1924	TCGAAAAGAGCG-AGGTCCCCCGGA	243.5 kb
361	18553-L23891	***NUP214*, ex 2**	206-207	AGAATCTTTGAC-TCCCCTGAGGAA	47.9 kb
492	18547-L24044	***NUP214*, ex 23**	3296-3297	TCTGCTAGCAAA-ATTATTCCTCAA	-

***PTEN* gene**, at 10q23.31, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_000314.8.
PTEN deletions occurring in 9% of T-ALL patients are associated with early treatment failure and may contribute to increased resistance to chemotherapy (Gutierrez et al. 2009; Jotta et al. 2010). *PTEN* mutations in T-ALL seem to cluster in exon 7 (Zuurbier et al. 2012).

233	13685-L17760	***PTEN*, ex 1**	781-782	CCTGCAGAAGAA-GCCCCGCCACCA	93.5 kb
214 #	13691-L23847	***PTEN*, ex 7**	1550-1551	ACACGACGGGAA-GACAAGTTCATG	0.1 kb
439	13692-L23864	***PTEN*, ex 7**	4 nt after ex 7	TAAAAAAGGTTT-GTACTTTACTTT	7.6 kb

475 #	13696-L17520	***PTEN*, ex 9**	2171-2170 reverse	AGAGAATTGTTC-CTATAACTGGTA	-
***LMO1* gene**, at 11p15.4, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_002315.3. *LMO1* is a transcriptional regulator, which is involved in translocations between *TRB* (7q34) and *TRA/D* (14q11) leading in overexpression of *LMO1*. *LMO1* has been recognized to be amplified and to act as an oncogene in neuroblastoma (Wang et al. 2011). Co-expression of *LMO1* and *TAL1* was shown to decrease disease latency (Girardi et al. 2017).					
343 Ж	16717-SP0380-L19731	***LMO1*, ex 4**	935-934 and 899-898 reverse	CCTCATAGTCCA-36nt spanning oligo-TGTCTCCCACAC	5.7 kb
244 Ж	16714-SP0381-L23885	***LMO1*, ex 2**	634-633 and 598-597 reverse	GTACTTGTCCAA-36nt spanning oligo-ACAGCCCGCACA	26 **M**b
***RAG2-LMO2* fusion**, at 11p13, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_005574.4 for *LMO2* and NM_000536.4 for *RAG2*. Deletion of 11p13, including exon 1 of the *LMO2* gene, activates *LMO2* expression in 4% of paediatric T-ALL patients by fusing *RAG2* exon 1 to exon 2 of *LMO2* (Van Vlierberghe et al. 2006). ° A decreased signal of the probes for *LMO2* ex 1, *CD44* and *SLC1A2* is indicative for the formation of an *LMO2-RAG2* fusion.					
196	12538-L23846	***LMO2*, ex 6**	1459-1460	ACATAGCATCCA-AGTGGCATAATT	33.0 kb
382 °	12558-L23858	***LMO2*, ex 1**	77 nt before ex 1	AGCACATCTGGT-GTGAGAGAGCTC	1.3 **M**b
426 °	00669-L23838	***CD44***	11p13	CAGAAACTCCAG-ACCAGTTTATGA	36.4 kb
481 °	18698-L29605	***SLC1A2***	11p13	AGTGCATGAAGA-TATTGAAATGAC	1.3 **M**b
152	18551-L23889	***RAG2*, ex 2**	326-327	GCATAACCATGT-CAAACTGAAGCC	4.1 kb
351	16520-L23853	***RAG2*, ex 1**	97-98	GTTTAGCGGCAA-AGATTCAGAGAG	-
***NF1* and *SUZ12* genes**, at 17q11.2, indicated exon numbering is according to the MANE for both *NF1* and *SUZ12*. The indicated ligations sites are according to the LGR t1 transcript NM_000267.3 for *NF1* and MANE Select transcript NM_015355.4 for *SUZ12*. An *NF1* deletion detected in 11% of T-ALL patients may correlate with poor response to induction therapy (Balgobind et al. 2008; Matteucci et al. 2010). The *SUZ12* gene in the same chromosomal region has also been shown to be inactivated by loss-of-function mutations and deletions in T-ALL patients (Ntziachristos et al. 2012; Zhang et al. 2012).					
250 #	03849-L18072	***NF1*, ex 26**	3816-3817	TGAGGCACTGTA-CGGTCCTTGCAA	141.2 kb
400	02530-L23856	***NF1*, ex 58**	8748-8749	GCCACTGTAACA-GTGGACGAACTC	614.4 kb
504	18539-L23848	***SUZ12*, ex 10**	1407-1408	CATCAGGAAAAC-AAGCCTGGTTCA	8.4 kb
311	18705-L23805	***SUZ12*, ex 15**	2084-2085	AGAAGTGATGAA-ACTCTGGAATCT	-
***PTPN2* gene**, at 18p11.21, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_080422.4. A *PTPN2* deletion is detected in 6% of all T-ALL patients. *PTPN2* has been identified as a negative regulator of *NUP214-ABL1* and JAK1 kinase activity (Kleppe et al. 2010; Kleppe et al. 2011).					
462	15970-L23850	***PTPN2*, ex 9**	1380-1381	TCTGCACTAGTA-ACTGACAGTGCT	36.8 kb
208 Ж	18549-SP0318-L23866	***PTPN2*, ex 4**	363-364 and 390-391	TCACAGGGTCCA-27nt spanning oligo-CTTATGGTTTGG	28.2 kb
239 Ж	15955-SP0317-L18107	***PTPN2*, ex 2**	219-220 and 255-256	AAGTTTCCAGAA-36nt spanning oligo-TGTAAGTACTTG	25.0 kb
176 Ж ±	15951-SP0316-L23881	***PTPN2*, ex 1**	93-94 and 126-127	CTCGCTCCCGCA-33nt spanning oligo-GAGTTGGATACT	-
***PHF6* gene**, at Xq26.2, indicated ligation sites and exon numbering are according to the MANE Select transcript NM_001015877.2. *PHF6* inactivation is detected in 16% and 38% of paediatric and adult T-ALL cases, respectively. Inactivation occurs by deletion in 21% and by point mutation in 79% of inactivation cases, and correlates with poor survival in adult T-ALL (Van Vlierberghe et al. 2010).					
190 ¬	06104-L05559	*SH2D1A*	Xq25	TGAGCTCGTTTT-AACTGAAGTGTG	10 **M**b
137	18550-L29679	***PHF6*, ex 1**	110-111	TCTTTCTCTTTA-CCCTCATTGGCG	4.6 kb
161	15948-L23843	***PHF6*, ex 3**	377-378	CACACTCTGATA-ATGAAAGTCTTG	35.8 kb
226	15954-L23884	***PHF6*, ex 7**	823-824	AGCCCTAGTGAC-ACCAGGCCTAAA	12.1 kb
391	15965-L24481	***PHF6*, ex 11**	1649-1650	TTGTTTAGTGGA-TCCATACTCAAA	2.2 **M**b
202 ¬	02902-L02296	*ARHGEF6*	Xq26.3	GATGCTCAAATC-CTTAAAGTGATC	-

[a] See section Exon numbering on page 1 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.

± SNV rs200164592 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related products

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

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P383 product history	
Version	*Modification*
A2	Changes in length of several probes but no change in the sequences detected.
A1	First release.

Implemented changes in the product description

Version A2-06 – 28 July 2025 (04P)
- Description of the position of the 182 nt probe changed (no change in actual target site).
- Positive control DNA samples section: information moved to product page on website.
- Removed Related SALSA MLPA products section.

Version A2-05 – 07 February 2024 (04P)
- Exon numbering of the *TAL1* and *ABL1* genes has been changed according to MANE in Table 1 and 2. See section 'Exon numbering' on page 2 for more information.
- Ligation sites of the probes targeting the *TAL1, ABL1, RAG2* and *PTPN2* genes has been updated according to new version of the NM_ reference sequence.
- Removed the "« Probe located in or near a GC-rich region…" remark in Table 1 and 2.
- New selected publications for P383 added on page 12.
- Various minor textual changes and changes in layout.

Version A2-04 – 17 January 2023 (04P)
- Exon numbering of the *CDKN2A* gene has been changed according to MANE in Table 1 and 2. See also explanation on page 2.
- Ligation site of the CDKN2A probe 18753-L24594 has been updated.

Version A2-03 – 15 March 2021 (04P)
- Typo corrected in the title of the Table 2.
- P383 specific note added on page 4.
- New selected publication for P383 added on page 12.
- Order of the selected publications changed to alphabetical.

- Other minor typos corrected.

Version A2-02 – 19 January 2021 (04P)
- Positive sample table on page 3 adjusted: information changed for one sample and three positive samples removed upon critical review of the MLPA data with Coffalyser.Net software.

Version A2-01 – 05 January 2021 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *LEF1, CASP8AP2, MYB, EZH2, MLLT3, CDKN2A, NUP214, PTEN, LMO1, LMO2, RAG2, SUZ12, PTPN2* and *PHF6* genes 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.
- Warning added to Tables 1 and 2 for SNV rs200164592 in PTPN2 probe at 176 nt.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Reference probe function removed from MTTL3 and MTAP probes.

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