Product Description

SALSA® MLPA® Probemix P437-B1 Familial MDS-AML

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 10.

Catalogue numbers

- **P437-025R:** SALSA® MLPA® Probemix P437 Familial MDS-AML, 25 reactions
- **P437-050R:** SALSA® MLPA® Probemix P437 Familial MDS-AML, 50 reactions
- **P437-100R:** SALSA® MLPA® Probemix P437 Familial MDS-AML, 100 reactions

SALSA® MLPA® Probemix P437 Familial MDS-AML (hereafter: P437 Familial MDS-AML) 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.)

P437 Familial MDS-AML can be used in combination with:

- SALSA® Binning DNA SD070 (Cat. No: SD070)

Volumes and ingredients

Volumes			Ingredients
P437-025R	**P437-050R**	**P437-100R**	
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 P437 Familial MDS-AML is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GATA2* (3q21.3), *TERC* (3q26.2), *TERT* (5p15.33), *CEBPA* (19q13.11) and *RUNX1* (21q22.12) genes, which are suggested to be of diagnostic relevance in familial MDS and AML. This probemix can also be used to detect the presence of three mutations, namely, the *GATA2* p.R398W (c.1192C>T), *GATA2* p.T354M (c.1061C>T) and *TERT* p.A1062T (c.3184G>A).

While the majority of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) cases are sporadic, familial MDS and AML cases have also been identified and included as a separate disease entity in World Health Organization (WHO) classification of hematological cancers. Inherited mutations in the *GATA2*, *TERC*, *TERT*, *CEBPA* and *RUNX1* genes have been shown to associate with familial MDS and AML (for review see Holme et al. 2012). Although most of the germline aberrations in these genes are point mutations, deletions have been described as well, e.g. in the *GATA2* and *RUNX1* genes (Hsu et al. 2011, Kazenwadel et al. 2012, and Liew & Owen 2011). The most recurrent *GATA2* mutations identified in MonoMAC patients are p.R398W (c.1192C>T) and p.T354M (c.1061C>T) (Hsu et al. 2011). Furthermore, in the *TERT* gene the p.A1062T (c.3184G>A) mutation is shown to be a negative prognostic factor in younger AML patients (Both et al. 2017).

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
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE
Tark – Transcript Archive: https://tark.ensembl.org

Exon numbering

The *GATA2, TERT, CEBPA* and *RUNX1* exon numbering used in this P473-B1 Familial MDS-AML product description is the exon numbering derived from MANE project (release version 1.3) based on respective MANE Select transcripts (See Table 2 for details). The *TERC* exon numbering is based on the NR_001566.1, which is the reference standard in the NCBI RefSeqGene project. From description version B1-02 onwards, the *GATA2* exon numbering has changed as we have adopted the MANE exon numbering. 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 in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content

The SALSA MLPA Probemix P478-B1 Familial MDS-AML contains 59 MLPA probes with amplification products between 124 and 505 nucleotides (nt). This includes 42 probes for the genes *GATA2*, *TERC*, *TERT*, *CEPBA* and *RUNX1*. Furthermore, this probemix also contains three probes specific for the *GATA2* p.R398W, *GATA2* p.T354M and *TERT* p.A1062T point mutations, which will only generate a signal when the mutation is present. In addition, 14 reference probes are included that target relatively copy number stable regions in various cancer types including familial MDS-AML. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 3 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 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 AML or MDS. 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. Samples from the Coriell Institute and DSMZ have been tested with P437-B1 probemix at MRC Holland and can be used as a positive control samples as described in the table below. 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.

Sample name	Source	Chromosomal position (hg18) of CNA*	Altered target genes in P437-B1	Expected CNA
NA10175	Coriell Institute	3q26.2	*TERC*	Heterozygous duplication
NA11428				
NA20022				
NA14523	Coriell Institute	5p15.33	*TERT*	Heterozygous duplication
NA14131	Coriell Institute	5p15.33	*TERT*	Heterozygous deletion

NA01201	Coriell Institute	21q22.12	*RUNX1*	Heterozygous deletion
MFE-280◊	Leibniz Institute DSMZ	3q21.3	*GATA2*	Gain
		3q26.2	*TERC*	Gain +
		5p15.33	*TERT*	Gain
		19q13.1	*CEBPA*	Gain +
SK-N-MC◊	Leibniz Institute DSMZ	3q21.3-q26.2	*GATA2 & TERC*	Gain

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by P437-B1 Familial MDS-AML probemix.
◊ In the indicated cell line samples some of the reference/digestion control/flanking probes are also affected by copy number alterations.
+ High signal, indicating amplification, was observed for probes targeting *TERC* and *CEBPA* genes.

SALSA® Binning DNA SD070
The SALSA® Binning DNA SD070 provided with this probemix can be used for binning of all probes including the three mutation-specific probes *GATA2* probe 19052-SP0847-L24939 (p.R398W=c.1192C>T) at 168 nt, *GATA2* probe 19053-SP0738-L25512 (p.T354M=c.1061C>T) at 190 nt and *TERT* probe 19697-SP0859-L30075 (p.A1062T=c.3184G>A) at 200 nt. SD070 Binning DNA 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 SD070 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. For further details, please consult the SD070 Binning DNA 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 in germline analysis. If P437 probemix is used for somatic analysis, reference probes in the patient samples might have final ratios outside of 0.80 and 1.20 if the target sequence of reference probe has a gain or a loss. When these criteria are fulfilled, the following cut-off values for the finale ratio (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.

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 mosaic or subclonal cases. Analysis of parental samples may be necessary for correct interpretation of complex germline 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 or in or near the *RUNX1* and *CEBPA* genes. 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 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.

P437 specific notes

- 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.
- The *GATA2* and *TERT* mutation-specific probes are only intended to determine the presence (or absence) of the mutation.

Limitations of the procedure

- In most populations, the major cause of germline genetic defects in the *GATA2, TERC, TERT, CEBPA or RUNX1* genes are small (point) mutations, most of which will not be detected by using P437 Familial MDS-AML. The two and one most common point mutations in the *GATA2* and *TERT* genes, respectively, can be detected, but other point mutations in the *GATA2* and *TERT* 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.
- 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 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.

LOVD mutation database

https://databases.lovd.nl/shared/genes/GATA2
https://databases.lovd.nl/shared/genes/TERC
https://databases.lovd.nl/shared/genes/TERT
https://databases.lovd.nl/shared/genes/CEBPA
https://databases.lovd.nl/shared/genes/RUNX1

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

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *GATA2* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. P437-B1 Familial AML-MDS

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*GATA2*	*TERC*	*TERT*	*CEBPA*	*RUNX1*
64-105	Control fragments – see table in probemix content section for more information						
124	Reference probe 19616-L26241	4p13					
130	**TERC probe** 08640-L08656			**Exon 1**			
136	**CEBPA probe** 19049-L24936					**Exon 1**	
142	**GATA2 probe** 19050-L24937		**Exon 4**				
148	**TERT probe** 19696-L29831				**Exon 15**		
157	**RUNX1 probe** 19051-L25507						**Intron 7**
161	**TERT probe** 10346-L29829				**Exon 2**		
168 § Ж	**GATA2 probe** 19052-SP0847-L24939		**p.R398W= c.1192C>T**				
173	Reference probe 21100-L29832	1q32					
178	**TERT probe** 15272-L26713				**Exon 1**		
184	Reference probe 08731-L29833	9q21					
190 § Ж	**GATA2 probe** 19053-SP0738-L25512		**p.T354M= c.1061C>T**				
195	**TERT probe** 08647-L29834				**Exon 7**		
200 § Ж	**TERT probe** 19697-SP0859-L30075				**p.A1062T= c.3184G>A**		
207	**TERT probe** 19055-L30074				**Exon 6**		
212	**RUNX1 probe** 19057-L29836						**Exon 1**
219	**GATA2 probe** 19058-L25516		**Intron 1**				
224	**TERT probe** 19059-L25517				**Exon 9**		
229	**TERT probe** 08652-L25343				**Exon 12**		
233	Reference probe 15154-L25342	18q21					
241	**RUNX1 probe** 19014-L30135						**Exon 4**
246	**GATA2 probe** 21373-L30136		**Exon 6**				
253	Reference probe 10716-L30137	6p12					
258	**TERT probe** 19061-L30138				**Exon 4**		
265	**CEBPA probe** 19062-L24949					**Exon 1**	
270	**TERC probe** 19299-L24943			**Exon 1**			
276 Δ	**GATA2 probe** 19063-L30052		**Exon 5**				
283	**RUNX1 probe** 21375-L29826						**Exon 2**
288	Reference probe 02877-L24219	1p33					
296	**TERT probe** 19065-L26112				**Exon 3**		
301 «	**RUNX1 probe** 02840-L25520						**Exon 8**
309	**CEBPA probe** 19066-L26430					**Exon 1**	
316 «	**GATA2 probe** 21376-L29827		**Exon 1**				
324 «	**RUNX1 probe** 19017-L25523						**Exon 9**
331	**TERT probe** 08651-L26433				**Exon 11**		
337	Reference probe 20864-L28882	14q24					
346 Δ	**GATA2 probe** 19588-L26183		**Intron 1**				
355	**GATA2 probe** 21377-L29828		**Exon 2**				
360	**RUNX1 probe** 19069-L30005						**Exon 3**
366	**TERT probe** 08653-L30006				**Exon 13**		
373	**TERC probe** 19070-L30007			**Exon 1**			
381	Reference probe 19749-L26532	9q34					
388	**RUNX1 probe** 19019-L25441						**Exon 7**
395	**TERT probe** 08656-L25442				**Exon 16**		
402	Reference probe 01237-L25675	10p14					
409	**GATA2 probe** 19071-L24958		**Exon 3**				
418	**TERT probe** 19072-L24959				**Exon 10**		
425	**RUNX1 probe** 19237-L25317						**Exon 5**
431 Δ	Reference probe 15541-L25346	2q23					

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*GATA2*	*TERC*	*TERT*	*CEBPA*	*RUNX1*
439	**RUNX1 probe** 02838-L25676						**Exon 6**
445 Ø ∫	**GATA2 probe** 19298-L25506		**Intron 4**				
454	**TERT probe** 08645-L25504				**Exon 5**		
460	Reference probe 16287-L29839	20q11					
469	**TERT probe** 19073-L29840				**Exon 14**		
475	**TERT probe** 19074-L29841				**Exon 8**		
481	Reference probe 09772-L10187	15q21					
489 Ø	**TERC probe** 19295-L25478			**Upstream**			
497	Reference probe 19555-L30008	2p13					
505	Reference probe 06676-L29849	11p15					

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

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

§ Mutation-specific probe. This probe will only generate a signal when; in *GATA2* gene the p.R398W=c.1192C>T or p.T354M=c.1061C>T and in *TERT* gene the p.A1062T=c.3184G>A mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

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.

Ø Intron or flanking probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

∫ Important information on this probe can be found in and below Table 2.

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 hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. Target probes arranged according to chromosomal location

Length (nt)	MLPA probe	Exon[a] / (Mutation)	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
***GATA2* gene, at 3q21.3**. Ligation sites are indicated according to NM_032638.5, which is a MANE Select transcript. Ligations sites for intron 1 probes are indicated also according to the NM_001145661.2, which is a MANE Plus Clinical transcript. The *GATA2* exon numbering (LRG_295) used in previous versions of this product description can be found in between brackets.					
246	21373-L30136	Exon 6 (8)	1613-1614	AAAGTGCATGCA-GGAGAAGTCATC	0.1 kb
168 § Ж	19052-SP0847-L24939	Exon 6 (8) **c.1192C>T =p.R398W**	1540-1539 and 1504-1503 reverse	GGACATCTTCC**A**-36nt spanning oligonucleotide-TGGCCTGTTAAC	0.6 kb
276 Δ	19063-L30052	Exon 5 (7)	1491-1492	AAGCTGCACAAT-GTGAGTGCGCCC	0.1 kb
190 § Ж	19053-SP0738-L25512	Exon 5 (7) **c.1061C>T =p.T354M**	1409-1408 and 1383-1382, reverse	TGGTGGTTGTC**A**-26nt spanning oligonucleotide-GCTCTTCTGGCG	1.4 kb
445 Ø ∫	19298-L25506	Intron 4 (6)	0.5 kb after ex 4	ACATCTGCAGCC-TGAAGATAAGGA	0.6 kb
142	19050-L24937	Exon 4 (6)	1341-1342	CAGAACCGACCA-CTCATCAAGCCC	2.5 kb
409	19071-L24958	Exon 3 (5)	597-598	ACCGGAGGCCAG-ATGTGCCGCCCA	0.5 kb
355	21377-L29828	Exon 2 (4)	513-514	CTCGACTCGCAG-GGCAACCCCTAC	0.9 kb
219	19058-L25516	Intron 1 (ex 3)	0.7 kb before ex 2; NM_001145661.2; 355-356	CCTTCACTCTCA-GAGGCCGAGTCC	0.6 kb
346 Δ	19588-L26183	Intron 1 (ex 2)	1.3 kb before ex 2; NM_001145661.2; 166-167	CGAGCTAGGGGA-GGGAACGGTCTG	4.8 kb

Length (nt)	MLPA probe	Exon[a] / (Mutation)	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
316 «	21376-L29827	Exon 1	28-29	GAGCGCCAGGAA-GGTAGCGAGGCC	41.3 **M**b to *TERC*
***TERC* gene, at 3q26.2.** Ligation sites are indicated according to NR_001566.1, which is a reference standard in the NCBI RefSeqGene project.					
270	19299-L24943	Exon 1	100 nt after ex 1, reverse	TGCAGCACACTG-GCCCAGTCAGTC	0.2 kb
130	08640-L08656	Exon 1	314-315	GAGTTGGGCTCT-GTCAGCCGCGGG	0.4 kb
373	19070-L30007	Exon 1	68 nt before ex 1, reverse	GGCCAAGCTGAC-TCTCGCGGCTCT	0.3 kb
489	19295-L25478	Upstream	0.4 kb before ex 1	GAGCCGAGACAA-GATTCTGCTGTA	-
***TERT* gene, at 5p15.33**. Ligation sites are indicated according to NM_198253.3, which is a MANE Select transcript.					
395	08656-L25442	Exon 16	13 nt before ex 16	ACGGAGTCTGAT-TTTGGCCCCGCA	0.5 kb
148	19696-L29831	Exon 15	3364-3365	CTCCTGGGGTCA-CTCAGGACAGGC	0.1 kb
200 § Ж	19697-SP0859-L30075	Exon 15, **c.3184G>A =p.A1062T**	3263-3264 and 3291-3292	CCAAGGGCGCC**A**-28nt spanning oligonucleotide-GTGGCTGTGCCA	0.8 kb
469	19073-L29840	Exon 14	3228-3229	CCTGAAAGCCAA-GAACGCAGGTAT	3.3 kb
366	08653-L30006	Exon 13	3083-3084	GCACCAACATCT-ACAAGATCCTCC	1.9 kb
229	08652-L25343	Exon 12	3000-3001	GCGTCGCAAACT-CTTTGGGGTCTT	4.0 kb
331	08651-L26433	Exon 11	2809-2810	TTCCCTGTAGAA-GACGAGGCCCTG	1.9 kb
418	19072-L24959	Exon 10	1 nt after ex 10	ACCTTCCTCAGG-TGAGGCCCGTGC	2.0 kb
224	19059-L25517	Exon 9	99 nt after ex 9	ACCATGACTGCT-CTGTCTTGAGGA	2.8 kb
475	19074-L29841	Exon 8	3 nt before ex 8	CGTCTGCTTTCG-CAGAGCTCCTCC	1.0 kb
195	08647-L29834	Exon 7	2405-2406	ACATGCGACAGT-TCGTGGCTCACC	6.4 kb
207	19055-L30074	Exon 6	2345-2344, reverse	GAAGGCCTTGCG-GACGTGCCCATG	0.7 kb
454	08645-L25504	Exon 5	2134-2135	GGCCTGGACGAT-ATCCACAGGGCC	1.0 kb
258	19061-L30138	Exon 4	70 nt before ex 4	GTGGGGGCTTGT-GGCTTCCCGTGA	2.2 kb
296	19065-L26112	Exon 3	1700-1701	GTGAGGAGATCC-TGGCCAAGTTCC	12.1 kb
161	10346-L29829	Exon 2	321-322	GAAGGAGCTGGT-GGCCCGAGTGCT	0.3 kb
178	15272-L26713	Exon 1	152-153	TGCTGCCGCTGG-CCACGTTCGTGC	-
***CEBPA* gene, at 19q13.11**. Ligation sites are indicated according to NM_004364.5, which is a MANE Select transcript.					
265	19062-L24949	Exon 1	743-744	GCACCTGCAGTT-CCAGATCGCGCA	0.4 kb
136	19049-L24936	Exon 1	359-360	GTTCCTGGCCGA-CCTGTTCCAGCA	0.2 kb
309	19066-L26430	Exon 1	114-115	GAGAACTCTAAC-TCCCCCATGGAG	-
***RUNX1* gene, at 21q22.12**. Ligation sites are indicated, unless noted otherwise, according to NM_001754.5, which is a MANE Select transcript.					
324 «	19017-L25523	Exon 9	1409-1410	TCCTACCACCTG-TACTACGGCGCC	7.1 kb
301 «	02840-L25520	Exon 8	1040-1041	TGGTCCTACGAT-CAGTCCTACCAA	22.3 kb
157	19051-L25507	Intron 7	12.7 kb after ex 7; NM_001122607.2: 2321-2322	AGAGGAAGACAC-AGCACCCTGGAG	12.8 kb
388	19019-L25441	Exon 7	982-981, reverse	TCTGACTCTGAG-GCTGAGGGTTAA	25.1 kb
439	02838-L25676	Exon 6	743-744	TTCACAAACCCA-CCGCAAGTCGCC	21.1 kb
425	19237-L25317	Exon 5	627-628	CGGCTGAGCTGA-GAAATGCTACCG	6.5 kb
241	19014-L30135	Exon 4	294-295	TCCCCGTAGATG-CCAGCACGAGCC	5.8 kb
360	19069-L30005	Exon 3	284-285	CCTTCTAGAGAC-GTCCACGGTATG	155.9 kb
283	21375-L29826	Exon 2	239-240	TCGTACCCACAG-TGCTTCATGAGA	0.4 kb
212	19057-L29836	Exon 1	75-74, reverse	CTGACCACTATG-CTGGGTTCAGAC	-

[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] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

§ Mutation-specific probe. This probe will only generate a signal when the described mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

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.

Ø Intron or flanking probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

∫ This probe covers a conserved intronic element (consisting of E-box and GATA motifs) that is under haploinsufficiency suggested to lead to MonoMAC syndrome (see Hsu et al. 2013).

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 3. Reference probes arranged according to chromosomal location.

Length (nt)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
288	02877-L24219	*FAF1*	1p33	GGACCTGCATTT-AATCCAGCAAGT	01-051,026
173	21100-L29832	*CACNA1S*	1q32	AAGCCGCCATGA-AGATCATTGCCT	01-199,330
497	19555-L30008	*DYSF*	2p13	CCATTGCCAAGA-AGGTCAGTGTCC	02-071,750
431 Δ	15541-L25346	*ACVR2A*	2q23	TGTGTAGGTGAA-AGAATTACCCAG	02-148,401
124	19616-L26241	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
253	10716-L30137	*PKHD1*	6p12	GCCATCCTTGTT-TCTGATGGTGGA	06-051,907
184	08731-L29833	*PCSK5*	9q21	GTCCTGGATTCT-ATGGTGACCAAG	09-078,101
381	19749-L26532	*STXBP1*	9q34	CAAAGCTTCTAC-AGTCCCCACAAG	09-129,465
402	01237-L25675	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
505	06676-L29849	*SMPD1*	11p15	CTGCTGAAGATA-GCACCACCTGCC	11-006,369
337	20864-L28882	*PSEN1*	14q24	TTTCTGTGAAAC-AGTATTTCTATA	14-072,684
481	09772-L10187	*SPG11*	15q21	TTTCTTCAGGAT-TGATAGTCATTC	15-042,706
233	15154-L25342	*MC4R*	18q21	GACATTTACTCA-CAGCAGGCATGG	18-056,191
460	16287-L29839	*SAMHD1*	20q11	CCGACTACAAGA-CATGGGGTCCGG	20-035,013

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

Complete probe sequences are available at www.mrcholland.com.

Related products

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

References

- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- Both A et al. (2017). The hypomorphic TERT A1062T variant is associated with increased treatment-related toxicity in acute myeloid leukemia. *Ann Hematol*. 96:895-904.
- Holme H et al. (2012). Marked genetic heterogeneity in familial myelodysplasia/acute myeloid leukaemia. *Br J Haematol.* 158:242-8.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Hsu AP et al. (2011). Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood*. 118:2653-5.
- Kazenwadel J et al. (2012). Loss-of-function germline GATA2 mutations in patients with MDS/AML or MonoMAC syndrome and primary lymphedema reveal a key role for GATA2 in the lymphatic vasculature. *Blood*. 119:1283-91.
- Liew E and Owen C (2011). Familial myelodysplastic syndromes: a review of the literature. *Haematologica*. 96:1536-42.

- 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 P437 Familial MDS-AML

- Almazni I et al. (2022). A novel RUNX1 exon 3-7 deletion causing a familial platelet disorder. *Platelets*. 33:320-3.
- Banescu C et al. (2019). Presence of copy number aberration and clinical prognostic factors in patients with acute myeloid leukemia: an analysis of effect modification. *Pol Arch Intern Med*. 23:898-906.
- Cheng CK et al. (2023). Deep genomic characterization highlights complexities and prognostic markers of pediatric acute myeloid leukemia. *Commun Biol*. 6: 356.
- Chicano M et al. (2021). Next generation cytogenetics in myeloid hematological neoplasms: Detection of CNVs and translocations. *Cancers (Basel).* 13:3001.
- Duployez N et al. (2019). Germline RUNX1 Intragenic Deletion: Implications for Accurate Diagnosis of FPD/AML. *Hemasphere*. 3: e203.
- de Andrade Silva MC et al. (2018). Deletion of RUNX1 exons 1 and 2 associated with familial platelet disorder with propensity to acute myeloid leukemia. *Cancer Genetics*. 222–223:32-7.
- Eriksson A et al. (2023). Somatic exonic deletions in RUNX1 constitutes a novel recurrent genomic abnormality in acute myeloid leukemia. *Clin Cancer Res.* 29:2826-34.
- Engvall M et al. (2022). Familial platelet disorder due to germline exonic deletions in RUNX1: a diagnostic challenge with distinct alterations of the transcript isoform equilibrium. *Leuk Lymphoma*. 63:2311-20.
- Largeaud L et al. (2023). Somatic genetic alterations predict hematological progression in GATA2 deficiency. *Haematologica*. 108:1515-29.
- Rio-Machin A et al. (2020). The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. *Nat Commun*. 11:1044.

P437 product history	
Version	*Modification*
B1	A new mutation-specific probe for *TERT* has been included, five target probes have been replaced, one new reference probe has been included, six reference probes have been replaced and several probes have been adjusted in length.
A1	First release.

Implemented changes in the product description
Version B1-02 – 24 May 2024 (05P) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Added a P437 specific note on page 5. - Exon numbering of the *GATA2* gene has been changed according to MANE Select. - Ligation sites of the probes targeting the *GATA2* gene updated according NM_032638.5 (MANE Select transcript). - New references added in section 'selected publications' on page 11. Version B1-01 – 18 May 2021 (04P) - Product description rewritten and adapted to a new template. - Warning added under Table 1 and 2b for 15541-L25346, 431 nt probe and 19063-L30052, 276 nt probe. - Positive samples added on page 3. - Ligation sites of the probes targeting the *GATA2, TERT, CEBPA* and *RUNX1* genes updated according to newest versions of the NM_ reference sequences. - New references added in section 'selected publications' on page 10.

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