

Product Description

SALSA® MLPA® Probemix P239-C1 BRCA1 region

To be used with the MLPA General Protocol.

Version C1

As compared to version B1, one flanking probe targeting the *STAT3* gene and three reference probes have been replaced. For complete product history see page 8.

Catalogue numbers:

- **P239-025R:** SALSA MLPA Probemix P239 BRCA1 region, 25 reactions.
- **P239-050R:** SALSA MLPA Probemix P239 BRCA1 region, 50 reactions.
- **P239-100R:** SALSA MLPA Probemix P239 BRCA1 region, 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 P239 BRCA1 region is a **research use only (RUO)** assay for the detection of deletions or duplications in the *BRCA1* region, which is associated with hereditary breast and ovarian cancer.

Defects in the *BRCA1* gene on human chromosome 17q21 are an important cause of hereditary breast and ovarian cancer. This SALSA MLPA Probemix P239 BRCA1 region can be used to characterise deletions/duplications that extend to the region up- and downstream of the *BRCA1* gene. For primary screening of *BRCA1*, we recommend using SALSA MLPA Probemix P002 BRCA1. *BRCA1* deletions and duplications detected using SALSA MLPA Probemix P002 BRCA1 can be confirmed with SALSA MLPA probemix P087 BRCA1 Confirmation. SALSA MLPA Probemix P239 BRCA1 region is developed for research purposes to investigate the extent of *BRCA1* deletions/duplications.

More information is available at <https://www.ncbi.nlm.nih.gov/books/NBK1247/>

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:

Entrez Gene shows transcript variants of each gene: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>

For NM_ mRNA reference sequences: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide>

Locus Reference Genomic (LRG) database: <http://www.lrg-sequence.org/>

Exon numbering

- The *BRCA1* exon numbering used in this P239-C1 BRCA1 region product description is the traditional exon numbering (exons 1a, 1b, 2, 3 and 5-24), wherein no exon 4 is present. **Please note that the *BRCA1* exon numbering in the *BRCA1* LRG 292 sequence and in the NCBI NG 005905.2 reference sequence is different. In Table 1 and Table 2 the LRG exon numbering is indicated between brackets.**
- The *GRN*, *G6PC1* and *STAT3* exon numbering used is the exon numbering from the LRG_661, LRG_147 and LRG_112 sequences, respectively.

- The exon numbering used for the long non-coding RNA *NBR2* is from the NR_003108.2 sequence.
- The exon numbering used for the *BRCA1P1*, *RND1* and *VAT1* genes is the exon numbering from the RefSeq transcript NM_ sequences (Table 2).
- The *NBR1* exon numbering is based on the combination of the NM_ sequences of the different *NBR1* transcript variants.
- The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG or NG sequences or the combination of the NM_ sequences of different transcript variants. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date

Probemix content

The SALSA MLPA Probemix P239-C1 *BRCA1* region contains 32 MLPA probes with amplification products between 130 and 400 nucleotides (nt). This includes 11 probes for the upstream region of the *BRCA1* gene, including probes for the *GRN*, *NBR1* and *NBR2* genes, and the *BRCA1* pseudogene *BRCA1P1*. Furthermore, five probes for the *BRCA1* gene and six probes for the downstream region of the *BRCA1* gene are included, including probes for the *G6PC1*, *RND2*, *STAT3* and *VAT1* genes. 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 the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation

Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤ 0.10 for all probes over the experiment.

Required specimens

Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples

A sufficient number (≥ 3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of hereditary breast and ovarian cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (<https://catalog.coriell.org>) and Leibniz Institute DSMZ (<https://www.dsmz.de/>) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤ 0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	$0.80 < FR < 1.20$
Homozygous deletion	FR = 0
Heterozygous deletion	$0.40 < FR < 0.65$
Heterozygous duplication	$1.30 < FR < 1.65$
Heterozygous triplication/homozygous duplication	$1.75 < FR < 2.15$
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: <http://dgv.tcag.ca/dgv/app/home>. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.

- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Notes *BRCA1* results:

- A heterozygous deletion of one or more *BRCA1* exons that are present in the major transcript variant NM_007294.4, including the non-coding exon 1a, is expected to result in a hereditary predisposition to breast cancer. The clinical significance of a deletion of only *BRCA1* exon 1b, which is not present in major transcript variant 1 (NM_007294.4), is not clear.
- Deletions of *BRCA1* exon 1b are relatively frequent and are part of a deletion encompassing exons 1a, 1b and 2 (van den Ouweland et al. 2009). Lower probe signals for the exon 1b probe should be treated with caution though, as the presence of salt in the DNA sample can lead to incomplete DNA denaturation, especially of the GC-rich region near exon 1b.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *BRCA1* region are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P239 *BRCA1* region.
- 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 solid 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.

BRCA1 mutation database

<https://databases.lovd.nl/shared/genes/BRCA1>. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on <http://varnomen.hgvs.org/>.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P239-C1 BRCA1 region

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a		
		Reference	BRCA1	BRCA1 region
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 01212-L00766	8p		
136	NBR2 probe 08348-L08280			NBR2 Exon 3
148	BRCA1P1 probe 08338-L08270			BRCA1P1 upstream
157 « » +	BRCA1 probe 00764-L00269		Exon 1b	
173	NBR1 probe 08344-L08276			NBR1 Exon 3
184	Reference probe 04589-L03798	3q		
190	G6PC1 probe 16983-L07227			G6PC1 Exon 4
202	Reference probe 13049-L14232	15q		
208 »	BRCA1 probe 00827-L00342		Exon 6 (5)	
214	Reference probe 11396-L12122	1q		
220	NBR2 probe 08349-L09138			NBR2 Exon 5
226	BRCA1P1 probe 08339-L08271			BRCA1P1 upstream
232 «	VAT1 probe 08335-L19990			VAT1 Exon 6
239 *	Reference probe 21060-L29289	10q		
247	BRCA1 probe 16984-L13862		Exon 24 (23)	
256 «	RND2 probe 08336-L08268			RND2 Exon 2
265	NBR1 probe 08345-L08277			NBR1 Exon 7
274 «	GRN probe 10172-L10625			GRN Exon 12
285 »	BRCA1 probe 00775-L00280		Exon 12 (11)	
292	Reference probe 07154-L06766	19q		
301	NBR1 probe 16987-L08278			NBR1 Exon 11
310	NBR2 probe 16988-L08279			NBR2 Exon 1
318 *	Reference probe 22004-L30847	11q		
328 «	GRN probe 10171-L21353			GRN Exon 10
337 »	BRCA1 probe 00779-L00003		Exon 16 (15)	
344 *	STAT3 probe 16095-L18265			STAT3 Exon 7
352	Reference probe 13681-L15146	5q		
364 «	RND2 probe 08337-L08269			RND2 Exon 4
373 «	VAT1 probe 16986-L08266			VAT1 Exon 2
382 *	Reference probe 13329-L14755	18q		
391	BRCA1P1 probe 08341-L08273			BRCA1P1 upstream
400	Reference probe 09109-L09168	4q		

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

* New in version C1.

« 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.

» Detects the same sequence as a probe in SALSA MLPA Probemix P002-D1 BRCA1.

+ This probe is located within *BRCA1* exon 1b, which is in close proximity to exon 1a of the major *BRCA1* transcript variant 1 (NM_007294.4). The clinical relevance of *BRCA1* exon 1b deletions/duplications is currently unclear.

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. BRCA1 region probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene exon ^a	Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
344	16095-L18265	STAT3 exon 7	NM_139276.3; 767-768	GAAACAACCAGT-CAGTGACCAGGC	571.6 kb
190	16983-L07227	G6PC1 exon 4	NM_000151.4; 2 nt after exon 4	TCCTGTCAGGTA-TGGGCTGATCTG	106.7 kb
232 «	08335-L19990	VAT1 exon 6	NM_006373.4; 1193-1194	GATGCAGGAGAA-GAAGAATGTGGG	2.6 kb
373 «	16986-L08266	VAT1 exon 2	NM_006373.4; 554-555	CATGACCTTTGA-GGAAGCTGCTGC	7.3 kb
256 «	08336-L08268	RND2 exon 2	NM_005440.5; 282-283	AAGCGCCGCATT-GAGCTCAACATG	2.1 kb
364 «	08337-L08269	RND2 exon 4	NM_005440.5; 476-477	GGTTGGCTGTAA-ACTGGACATGCC	17.1 kb
247	16984-L13862	BRCA1 exon 24 (23)	NM_007294.4; 6155-6156	TCAATGGAAGGA-GAGTGCTTGGGA	26.0 kb
337 »	00779-L00003	BRCA1 exon 16 (15)	NM_007294.4; 4819-4820	ATCTGGAATCAG-CCTCTTCTCTGA	19.8 kb
285 »	00775-L00280	BRCA1 exon 12 (11)	NM_007294.4; 4258-4259	CTCTGAAGACTG-CTCAGGGCTATC	13.9 kb
208 »	00827-L00342	BRCA1 exon 6 (5)	NM_007294.4; 354-355	CGAGATTTAGTC-AACTTGTTGAAG	20.1 kb
157 « » +	00764-L00269	BRCA1 exon 1b	Exon 1b; NM_007294.4 208 nt after exon 1a	AGGGGGCACTGA-GTGTCCGTGGGG	0.6 kb
310 #	16988-L08279	NBR2 exon 1	NR_003108.2; 103-104	CGGCTTATTGCA-TCACAGTAATTG	13.1 kb
136 #	08348-L08280	NBR2 exon 3	NR_003108.2; 340-339 reverse	CACGTGACTACC-TTTTATTGGCAG	6.3 kb
220 #	08349-L09138	NBR2 exon 5	NR_003108.2; 1307-1308	GAGATCAGCGCA-ATTGTCAGCTAA	23.2 kb
391 #	08341-L08273	BRCA1P1 upstream	NM_031862.4; 2175 nt before NBR1 reverse	ATTTATCTGCTG-TTTGCGTTGAAG	0.9 kb
226 #	08339-L08271	BRCA1P1 upstream	NM_031862.4; 1278 nt before NBR1 reverse	GCGGAAGAAGAA-GTGCCAGGAATA	0.5 kb
148 #	08338-L08270	BRCA1P1 upstream	NM_031862.4; 732 nt before NBR1 reverse	TTTCCGAAGCTA-GGCAGATGGGTA	6.2 kb
173	08344-L08276	NBR1 exon 3	NM_031862.4; 302-303	ACTTGGGCTGAT-ATCGAAGCTATG	10.5 kb
265	08345-L08277	NBR1 exon 7	NM_031862.4; 552-553	CACTTGACATT-ACTTCTACTGG	5.0 kb
301	16987-L08278	NBR1 exon 11	NM_031862.4; 1095-1096	AACAGGTTGATA-AGAAGTTCTTA	1187.6 kb
328 «	10171-L21353	GRN exon 10	NM_002087.4; 1113-1114	GCCAGACCCACA-AGCCTGAAGAG	0.8 kb
274 «	10172-L10625	GRN exon 12	NM_002087.4; 1616-1617	GGAAGGACACT-TCTGCCATGATA	

^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.

« 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.

» Detects the same sequence as a probe in SALSA MLPA Probemix P002-D1 BRCA1.

+ This probe is located within *BRCA1* exon 1b, which is in close proximity to exon 1a of the major *BRCA1* transcript variant 1 (NM_007294.4). The clinical relevance of *BRCA1* exon 1b deletions/duplications is currently unclear.

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. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P002 BRCA1	Contains probes for the <i>BRCA1</i> gene, involved in breast and ovarian cancer. It should be used for primary screening of <i>BRCA1</i> .
P087 BRCA1 Confirmation	Contains probes for the <i>BRCA1</i> gene. It can be used to confirm the results obtained with P002.
P045 BRCA2/CHEK2	Contains probes for the <i>BRCA2</i> and <i>CHEK2</i> genes, involved in breast and ovarian cancer.
P090 BRCA2	Contains the same probes for the <i>BRCA2</i> gene as P045.
P077 BRCA2 Confirmation	Contains probes for the <i>BRCA2</i> gene. It can be used to confirm the results obtained with P045 or P090.
P041/P042 ATM	Contains probes for the <i>ATM</i> gene, involved in breast cancer and Ataxia Telangiectasia.
P056 TP53	Contains probes for the <i>TP53</i> gene, involved in Li-Fraumeni syndrome.
P057 FANCD2-PALB2	Contains probes for the <i>FANCD2</i> and <i>PALB2</i> genes, involved in Fanconi Anemia.
P190 CHEK2	Contains probes for the <i>CHEK2</i> , <i>ATM</i> and <i>TP53</i> genes, involved in cancer.
P225 PTEN	Contains probes for the <i>PTEN</i> gene, involved in breast cancer.
P240 BRIP1/CHEK1	Contains probes for the <i>BRIP1</i> and <i>CHEK1</i> genes, involved in breast cancer.
P260 PALB2-RAD50- RAD51C-RAD51D	Contains probes for the <i>PALB2</i> , <i>RAD50</i> , <i>RAD51C</i> and <i>RAD51D</i> genes, involved in breast and ovarian cancer.

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.
- van den Ouweland AM et al. (2009). Deletion of exons 1a-2 of BRCA1: a rather frequent pathogenic abnormality. *Genet Test Mol Biomarkers.* 13:399-406.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P239 BRCA1 region

- Peixoto A et al. (2013). Genomic characterization of two large Alu-mediated rearrangements of the *BRCA1* gene. *J Hum Genet.* 58:78.

P239 product history	
Version	Modification
C1	One probe targeting the <i>STAT3</i> gene and three reference probes have been replaced.
B1	All target probes outside the <i>BRCA1</i> region have been removed and five target probes for the <i>BRCA1</i> region have been added. All reference probes have been replaced and QDX2 fragments have been added.
A1	First release.

Implemented changes in the product description

Version C1-02 – 15 February 2021 (04P)

- Product description adapted to a new template.
- Updated gene name *G6PC* to *G6PC1*.
- Notes on the interpretation of *BRCA1* deletions/duplications added.
- Link to *BRCA1* mutation database updated.
- *BRCA1* exon targeted by the 157 nt probe 00764-L00269 changed from exon 1a to exon 1b and clarification added about the *BRCA1* exon 1 designation.
- Exon numbering of the *BRCA1* LRG sequence added in brackets to Table 1 and 2.
- Ligation sites of the probes targeting the *BRCA1*, *BRCA1P1*, *GRN*, *G6PC1*, *NBR1* and *STAT3* genes updated according to new version of the NM_ reference sequence.
- Remark added to Table 1 and 2 about *BRCA1* probes also present in SALSA MLPA probemix P002-D1 *BRCA1*.
- SALSA MLPA probemixes P056 TP53 and P260 PALB2-RAD50-RAD51C-RAD51D added to the related probemix section.

Version C1-01 – 25 April 2019 (01P)

- Product description restructured 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 *VAT1*, *RND2*, *NBR2* and *NBR1* genes updated according to new version of the NM_ reference sequences.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 11 (55) – 07 March 2016

- Minor textual changes.
- Product description adapted to a new product lot (lot number added, new picture included).

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

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