Product Description
SALSA® MLPA® Probemix P193-B3 NPC1-NPC2-SMPD1

To be used with the MLPA General Protocol.

Version B3
As compared to version B2, five reference probes have been replaced. For complete product history see page 8.

Catalogue numbers:
- **P193-025R:** SALSA MLPA Probemix P193 NPC1-NPC2-SMPD1, 25 reactions.
- **P193-050R:** SALSA MLPA Probemix P193 NPC1-NPC2-SMPD1, 50 reactions.
- **P193-100R:** SALSA MLPA Probemix P193 NPC1-NPC2-SMPD1, 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 P193 NPC1-NPC2-SMPD1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *NPC1*, *NPC2* and *SMPD1* genes, which are associated with Niemann-Pick diseases. This probemix can also be used to detect the presence of the wild type sequences of the *NPC1* p.Pro1007Ala (exon 20) and the *NPC1* p.Ile1061Thr (exon 21) mutations.

Niemann-Pick diseases are lipid storage disorders that manifest in a wide range of symptoms with varying severity. Characteristic, however, are an accumulation of harmful quantities of lipids in the spleen, liver, lungs, bone marrow and brain. Niemann-Pick diseases are inherited in an autosomal recessive manner. Mutations in the *SMPD1* gene (chromosome 11p15.4) cause Niemann-Pick disease types A and B. Niemann-Pick type A occurs in infants and is characterised by hepatosplenomegaly, failure to drive and progressive deterioration of the nervous system. Niemann-Pick type B usually presents in mid-childhood. Signs and symptoms are comparable to Niemann-Pick type A, but are less severe. Moreover, patients often suffer from recurrent lung infections and thrombocytopenia.

Approximately 94% of Niemann-Pick type C cases are caused by mutations in the *NPC1* (90%, chromosome 18q11.2) and *NPC2* (4%, chromosome 14q24.3) genes. Niemann-Pick type C usually becomes apparent in mid-to-late childhood with onset of ataxia, vertical supranuclear gaze palsy and dementia.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1296/ (Niemann-Pick type C) and https://www.ncbi.nlm.nih.gov/books/NBK1370/ (Niemann-Pick types A and B)

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 exon numbering used in this P193-B3 NPC1-NPC2-SMPD1 product description is the exon numbering from the NG_012795.1 sequence for *NPC1*, NG_007117.1 for *NPC2*, and NG_011780.1 for *SMPD1*. 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 NG sequences. 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 P193-B3 NPC1-NPC2-SMPD1 contains 49 MLPA probes with amplification products between 130 and 493 nucleotides (nt). This includes 26 probes for the *NPC1* gene, five probes for the *NPC2* gene, and six probes for the *SMPD1* gene. Furthermore, this probemix also contains two probes detecting the wild type sequences of the *NPC1* p.Pro1007Ala (exon 20) and the *NPC1* p.Ile1061Thr (exon 21) mutations, which will only generate a signal when the mutation is present. 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 Niemann-Pick diseases. 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *NPC1*, *NPC2* and *SMPD1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P193 NPC1-NPC2-SMPD1.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Copy number changes detected by only a single probe 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.

Mutation databases

***NPC1*:** https://databases.lovd.nl/shared/genes/NPC1
***NPC2*:** https://databases.lovd.nl/shared/genes/NPC2
***SMPD1*:** https://databases.lovd.nl/shared/genes/SMPD1
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 (e.g., a duplication of *NPC1* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P193-B3 NPC1-NPC2-SMPD1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*NPC1*	*NPC2*	*SMPD1*
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 00797-L19287	5q			
138	**NPC1 probe** 12079-L12970		**Exon 6**		
143	**NPC1 probe** 10142-L26838		**Exon 15**		
149	**NPC1 probe** 10129-L11734		**Exon 3**		
154 *	Reference probe 18839-L24363	3p			
160	**NPC1 probe** 19465-L25879		**Exon 25**		
166	**SMPD1 probe** 19466-L25880				**Exon 5**
175	**NPC1 probe** 10143-L26841		**Exon 16**		
181	**SMPD1 probe** 19467-L26842				**Exon 1**
190	**NPC1 probe** 10128-L26904		**Exon 2**		
196	**NPC1 probe** 19468-L25882		**Exon 11**		
201	Reference probe 12681-L13759	7q			
208 *	Reference probe 05951-L21078	2p			
216	**NPC1 probe** 12394-L20060		**Exon 1**		
221	**NPC1 probe** 10154-L21611		**Exon 24**		
229	**NPC1 probe** 10149-L27417		**Exon 21**		
232	**NPC2 probe** 11660-L21613			**Exon 1**	
240	**SMPD1 probe** 19469-L26989				**Exon 6**
245	**NPC1 probe** 10145-L10607		**Exon 18**		
254 ∞ Ж	**NPC1 probe** 19740-SP0861-L26523		**Exon 20** p.Pro1007Ala		
261	**NPC1 probe** 10135-L27149		**Exon 8**		
267	**NPC2 probe** 11662-L26521			**Exon 4**	
275	**SMPD1 probe** 19498-L25950				**Exon 4**
283	**NPC1 probe** 21360-L21614		**Exon 17**		
290	**NPC1 probe** 19470-L26855		**Exon 1**		
297	**NPC1 probe** 10136-L26376		**Exon 9**		
303	Reference probe 15183-L16958	1q			
310	**NPC1 probe** 19472-L25886		**Exon 5**		
319 ∞	**NPC1 probe** 10151-L10615		**Exon 21** p.Ile1061Thr		
328	**NPC1 probe** 19474-L25888		**Exon 14**		
338	**NPC2 probe** 11663-L22048			**Exon 5**	
346 Ж	**NPC1 probe** 19499-SP0822-L27279		**Exon 23**		
355	**NPC1 probe** 10134-L10596		**Exon 7**		
362 *	Reference probe 19321-L25548	12p			
373	**NPC2 probe** 11664-L26850			**Exon 2**	
384	**NPC1 probe** 19477-L25891		**Exon 13**		
391	**NPC1 probe** 21361-L26251		**Exon 22**		
400	**NPC1 probe** 19500-L25952		**Exon 4**		
409 *	Reference probe 13405-L31765	6q			
418	**SMPD1 probe** 19479-L25893				**Exon 3**
427 *	Reference probe 15731-L17711	21q			
431	**NPC2 probe** 19501-L26903			**Exon 3**	
438	**NPC1 probe** 12652-L27418		**Exon 19**		
450	**NPC1 probe** 19481-L26852		**Exon 12**		
456	Reference probe 02144-L26853	13q			
463	**NPC1 probe** 19482-L25896		**Exon 10**		
475	**NPC1 probe** 19483-L25897		**Exon 20**		
484	**SMPD1 probe** 19484-L30069				**Exon 2**
493	Reference probe 09772-L22978	15q			

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

* New in version B3.
∞ Wild type sequence detected. A lowered probe signal can be due to a p.Pro1007Ala (254 nt probe, exon 20) or a p.Ile1061Thr (319 nt probe, exon 21) mutation, respectively. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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

Table 2. P193-B3 probes arranged according to chromosomal location

Table 2a. *SMPD1* gene at 11p15.4

Length (nt)	SALSA MLPA probe	*SMPD1* exon[a]	Ligation site NM_000543.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*126-128 (Exon 1)*		
181	19467-L26842	Exon 1	387-388	TTGGGTGGGGGA-ACCTCACCTGCC	1.2 kb
484	19484-L30069	Exon 2	1160-1161	TGGCTCTATGAA-GCGATGGCCAAG	1.3 kb
418	19479-L25893	Exon 3	1387-1388	TCGAGGAGACAA-AGTGAGGGCCAG	0.2 kb
275	19498-L25950	Exon 4	12 nt before exon 4	ACCATCCTTAAT-TCTCCCTACTAG	0.3 kb
166	19466-L25880	Exon 5	1515-1516	CTCATGTGGATG-AATTTGAGGTCT	0.7 kb
240	19469-L26989	Exon 6	2024-2025	TTTTGCTAGGGC-CCCAGGGCCCAC	
		stop codon	*2019-2021 (Exon 6)*		

Table 2b. *NPC2* gene at 14q24.3

Length (nt)	SALSA MLPA probe	*NPC2* exon[a]	Ligation site NM_006432.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*32-34 (Exon 1)*		
232	11660-L21613	Exon 1	17-18	CCGAGCTTGGAA-CTTCGTTATCCG	6.9 kb
373	11664-L26850	Exon 2	131-132	TGGATGGAGTTA-TAAAGGAAGTGA	1.9 kb
431	19501-L26903	Exon 3	261-262	GGTGCATGGCAT-CCTGATGGGCGT	3.8 kb
267	11662-L26521	Exon 4	433-434	CAGGATGACAAA-AACCAAAGTCTC	0.6 kb
338	11663-L22048	Exon 5	607-608	GTGATTCGTTGA-AGAGGAGGTGCT	
		stop codon	*485-487 (Exon 5)*		

Table 2c. *NPC1* gene at 18q11.2

Length (nt)	SALSA MLPA probe	*NPC1* exon[a]	Ligation site NM_000271.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*164-166 (Exon 1)*		
216	12394-L20060	Exon 1	214-215	CTACTGTGTCCA-GCGCAGGTGAGC	0.4 kb
290	19470-L26855	Exon 1	427 nt after exon 1	AGCAGAGGTAAA-GAGACACAAACT	12.3 kb
190	10128-L26904	Exon 2	266-267	GTGGAATTGCAT-ATGGGGACAAGA	1.4 kb
149	10129-L11734	Exon 3	413-414	TTCAGACACTAA-AAGACAACCTGC	3.3 kb
400	19500-L25952	Exon 4	583-584	ACGAAAACAAAT-GTGAAAGAGTTA	7.5 kb
310	19472-L25886	Exon 5	792-793	TCCTGTGTTTTC-AGGTAGGTATAA	1.0 kb
138	12079-L12970	Exon 6	974-975	CCATGTATGTCA-TCATGTGGATCA	3.1 kb
355	10134-L10596	Exon 7	1069-1068, reverse	TCGATGGGAGTG-TACTCGGAGACA	0.7 kb
261	10135-L27149	Exon 8	1290-1291	GGTCACAACCAA-TCCAGTTGACCT	1.5 kb
297	10136-L26376	Exon 9	1539-1540	CTCTTATGACAA-TGAGACTGTGAC	3.3 kb
463	19482-L25896	Exon 10	1806-1807	GCTTGTGTTGGG-AGGCTATGATGG	3.6 kb
196	19468-L25882	Exon 11	1919-1920	CCTGGGAAAAAG-AGTGAGTCACTC	2.9 kb
450	19481-L26852	Exon 12	1986-1987	TGCTGAACGAAG-TATTGAAGATGA	0.6 kb
384	19477-L25891	Exon 13	2193-2194	TGTCTTCAGCTA-CATTGGGTTGCC	1.0 kb
328	19474-L25888	Exon 14	2389-2390	TCCTTTTCTGAG-ACTGTAGCATTT	2.1 kb
143	10142-L26838	Exon 15	2471-2472	TGGCAGTCTTCA-TTGACTTTCTTC	0.3 kb
175	10143-L26841	Exon 16	2628-2629	TCGCTTCTTCAA-AAACTCCTATTC	0.6 kb
283	21360-L21614	Exon 17	2751-2750, reverse	GCATCGAAAGAG-ACTGATCCAATC	0.6 kb
245	10145-L10607	Exon 18	2858-2859	AAGGGCACGACT-ACACTTCTTCCA	0.5 kb
438	12652-L27418	Exon 19	2966-2967	CTAGTACCCGAA-TAGGCTTCGCCC	0.8 kb
475	19483-L25897	Exon 20	11 nt before exon 20	TCCTCCCTGCAT-GTCTCCGCCAGT	0.2 kb
254 ∞ Ж	19740-SP0861-L26523	Exon 20	p.Pro1007Ala 3182-3183 and 11 nt after exon 20	TTTCGGATAACC-33 nt spanning oligo-TGCCATTGCAGA	1.6 kb
229	10149-L27417	Exon 21	19 nt before exon 21	ACTCTCTTGACA-CCCAGGATTCTT	0.2 kb
319 ∞	10151-L10615	Exon 21	p.Ile1061Thr 3345-3344, reverse	CATTACTGGCTA-TAAGTCGGGCTT	1.2 kb
391	21361-L26251	Exon 22	3542-3543	CTGCAGTCATCA-TGTGTGCCACCA	1.0 kb
346 Ж	19499-SP0822-L27279	Exon 23	42 nt and 11 nt before exon 23	GCCTAGGGTCTT-31 nt spanning oligo-CTTTATTTCAGA	1.1 kb
221	10154-L21611	Exon 24	3793-3794	TTTGGAGGGATT-GTGGTGTTGGCT	1.3 kb
160	19465-L25879	Exon 25	4038-4037, reverse	AACCGACCGACC-CTTAGACACAGT	
		stop codon	*3998-4000 (Exon 25)*		

[a] See section Exon numbering on page 2 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.

∞ Wild type sequence detected. A lowered probe signal can be due to a p.Pro1007Ala (254 nt probe, exon 20) or a p.Ile1061Thr (319 nt probe, exon 21) mutation, respectively. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.

- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P193 NPC1-NPC2-SMPD1

- De Castro-Orós I et al. (2017). Assessment of plasma chitotriosidase activity, CCL18/PARC concentration and NP-C suspicion index in the diagnosis of Niemann-Pick disease type C: a prospective observational study. *J Trans Med.* 15:43.
- Hebbar M et al. (2016). Homozygous deletion of exons 2 and 3 of NPC2 associated with Niemann-Pick disease type C. *Am J Med Genet A*. 170:2486-2489.
- Jahnova H et al. (2014). Observational, retrospective study of a large cohort of patients with Niemann-Pick disease type C in the Czech Republic: a surprisingly stable diagnostic rate spanning almost 40 years. *Orphanet J Rare Dis.* 9:140.

P193 product history	
Version	*Modification*
B3	Five reference probes have been replaced.
B2	Two NPC1 and one SMPD1 probes have had a small change in length but not in sequence detected.
B1	Twelve NPC1 and NPC2 probes have been replaced and several NPC1 and NPC2 probes have had a small change in length but not in sequence detected. One NPC1 probe and six SMPD1 probes have been added. Several reference probes have been replaced and one reference probe has been added.
A2	Three reference probes and the 88 and 96 nt control fragments have been replaced. Several probes have had a small change in length but no change in sequence detected.
A1	First release.

Implemented changes in the product description
Version B3-01 – 11 January 2021 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *NPC1* and *SMPD1* genes updated according to new versions of the NM_ reference sequence. Version 08 – 30 May 2017 (55) - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). - New references added on page 2. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

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