Product Description SALSA® MLPA® Probemix P210-B1 BTK

To be used with the MLPA General Protocol.

Version B1. No changes.

Catalogue numbers:
- **P210-025R:** SALSA MLPA Probemix P210 BTK, 25 reactions.
- **P210-050R:** SALSA MLPA Probemix P210 BTK, 50 reactions.
- **P210-100R:** SALSA MLPA Probemix P210 BTK, 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.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P210-B1 BTK is a **research use only (RUO)** assay for the detection of deletions or duplications in the *BTK* gene, which is associated with Agammaglobulinemia.

Bruton's tyrosine kinase (BTK) is a non-receptor kinase that plays a crucial role in oncogenic signaling that is critical for proliferation and survival of leukemic cells in many B cell malignancies. BTK was initially shown to be defective in the primary immunodeficiency X-linked agammaglobulinemia (XLA) and is essential both for B cell development and function of mature B cells.

The *BTK* gene (19 exons), spans ~37 kb of genomic DNA and is located on chromosome Xq22.1, ~101 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1453/.

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 *BTK* exon numbering used in this P210-B1 BTK product description is the exon numbering from the RefSeq transcript NM_000061.2, which is identical to the LRG_128 sequence. The exon numbering and NM_ sequence used have been retrieved on 11/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P210-B1 BTK contains 29 MLPA probes with amplification products between 130 and 391 nucleotides (nt). This includes one probe for each exon of the *BTK* gene. Furthermore, this probemix contains probes for *GLA* and *RPL36A*, located approximately 15 kb upstream of BTK on Xq22.1. In addition, eight reference probes are included that detect other locations on the X-chromosome. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

This probemix contains ten quality control fragments generating amplification products between 64 and 121 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and two chromosome Y-specific fragments (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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific) (*AMOT* gene)
105	Y-fragment (Y chromosome specific) (*UTY* gene)
121	Y-fragment (Y chromosome specific) (*ZFY* gene)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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 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 unrelated individuals who are from families without a history of Agammaglobulinemia. 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.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.mlpa.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 dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results:

Copy number status: Male samples	Dosage quotient
Normal	0.80 < DQ < 1.20
Deletion	DQ = 0
Duplication	1.65 < DQ < 2.25
Ambiguous copy number	All other values

Copy number status: Female samples	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *BTK* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P210-B1 BTK.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

***BTK* mutation database:** https://databases.lovd.nl/shared/genes/BTK. 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 SNPs and unusual results (e.g., a duplication of *BTK* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P210-B1 BTK

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*BTK*
64-121	Control fragments – see table in probemix content section for more information		
130	Reference probe 13498-L06679	Xp22	
138	**BTK probe** 21137-L29375		**Exon 6**
152	**BTK probe** 06919-L29618		**Exon 12**
155	Reference probe 16067-L29646	Xq21	
164	**BTK probe** 21241-L29377		**Exon 1**
170	**BTK probe** 06924-L29376		**Exon 17**
176	**BTK probe** 06918-L06498		**Exon 11**
190	Reference probe 13201-L14522	Xq13	
196	**BTK probe** 21160-L29452		**Exon 2**
202	**BTK probe** 21161-L29980		**Exon 7**
211	**BTK probe** 21163-L29455		**Exon 13**
217	**BTK probe** 21164-L29456		**Exon 16**
224	**BTK probe** 21165-L29457		**Exon 19**
229 ¬	**GLA probe** 21166-L29617		**Upstream**
243	**BTK probe** 06910-L29378		**Exon 3**
250	**BTK probe** 06916-L29379		**Exon 9**
256	Reference probe 19937-L26978	Xq28	
265	**BTK probe** 06922-L06502		**Exon 15**
283	Reference probe 06793-L06385	Xq27	
301	**BTK probe** 21242-L29454		**Exon 8**
310	**BTK probe** 06921-L06501		**Exon 14**
319	Reference probe 10763-L11367	Xq23	
330 ¬	**RPL36A probe** 05174-L29380		**Upstream**
337	Reference probe 13107-L14326	Xq21	
349	**BTK probe** 06911-L29381		**Exon 4**
361	**BTK probe** 06912-L06492		**Exon 5**
373	**BTK probe** 06925-L06505		**Exon 18**
382	**BTK probe** 06917-L07130		**Exon 10**
391	Reference probe 03520-L02313	Xp11	

a) See above section on exon numbering for more information.

¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

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

Length (nt)	SALSA MLPA probe	Gene or *BTK* exon[a]	Ligation site NM_000061.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
229 ¬	21166-L29617	GLA		CCAGAGGGCCAT-CTGAGTTACTTG	2.7 kb
330 ¬	05174-L29380	RPL36A		AGAGCCATCAAT-ACAATTCCGCTT	9.7 kb
		Start Codon	*161-163 (exon 2)*		
164	21241-L29377	Exon 1	26-27	CTCTGGACTGTA-AGAATATGTCTC	10.9 kb
196	21160-L29452	Exon 2	187-188	CTGGAGAGCATC-TTTCTGAAGCGA	0.7 kb
243	06910-L29378	Exon 3	338-339	TAGATGTTGAGA-AGATCACTTGTG	2.9 kb
349	06911-L29381	Exon 4	443-444	AAATTTCAATCA-TTGAAAGGTTCC	1.6 kb
361	06912-L06492	Exon 5	494-495	AAGGGCCTCTCT-ACGTCTTCTCCC	7.4 kb
138	21137-L29375	Exon 6	615-616	CGATGGGCAGTA-TCTCTGCTGCTC	0.4 kb
202	21161-L29980	Exon 7	713-714	ACCGGAAGACAA-AAAAGCCTCTTC	1.5 kb
301	21242-L29454	Exon 8	831-830 reverse	TTGGCATGTAAT-CATAAAGGGCCA	0.6 kb
250	06916-L29379	Exon 9	958-959	TACATTCCTAGT-AACTATGTCACT	0.8 kb
382	06917-L07130	Exon 10	1016-1017	ATTCCAAACACA-TGACTCGGAGTC	0.6 kb
176	06918-L06498	Exon 11	1074-1075	AGGAGGTTTCAT-TGTCAGAGACTC	0.3 kb
152	06919-L29618	Exon 12	1191-1192	TCAGAGCCAGTA-TTACCTGGCTGA	0.8 kb
211	21163-L29455	Exon 13	1310-1309 reverse	AGTGGAAGGTGC-ATTCTTGTTTTG	0.7 kb
310	06921-L06501	Exon 14	1451-1452	TGGCCATCAAGA-TGATCAAAGAAG	0.7 kb
265	06922-L06502	Exon 15	1596-1597	GTACATGGCCAA-TGGCTGCCTCCT	1.5 kb
217	21164-L29456	Exon 16	1765-1766	CAAGGAGTTGTT-AAAGTATCTGAT	0.7 kb
170	06924-L29376	Exon 17	1800-1801	CAGGTATGTCCT-GGATGATGAATA	0.7 kb
373	06925-L06505	Exon 18	1962-1963	ATATGAGAGATT-TACTAACAGTGA	3.4 kb
224	21165-L29457	Exon 19	2171-2170 reverse	TTGTGGAGAAGA-GAAGTAGAACCA	
		Stop Codon	*2138-2140 (exon 19)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

Related SALSA MLPA probemixes

- P216 GHD: Contains probes for several genes involved in growth hormone deficiency.

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.

P210 Product history	
Version	*Modification*
B1	Six target probes, one flanking probe and seven reference probes have been replaced. One flanking probe has been removed and several probes have been adjusted in length.
A2	The 88, 96, 100 and 105 nt control fragments (QDX2) have been included. The 106 nt NPK0001 probe has been removed.
A1	First release.

Implemented changes in the product description

Version B1-01 — 02 December 2020 (02P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *BTK* gene updated according to new version of the NM_ reference sequence.

Version 13 – 11 May 2017 (55)
- Product description adapted to a new version (lot number added, small changes in Table 1 and Table 2, new pictures included).
- Various textual changes on page 1.

Version 12 – 12 July 2016 (55)
- Textbox added on page one.

Version 11 – 05 November 2015 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new pictures included).
- Various minor textual changes on page 1.

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