Product Description SALSA® MLPA® Probemix P205-B2 SH2D1A-XIAP-ITK

To be used with the MLPA General Protocol.

Version B2. For complete product history see page 7.

Catalogue numbers:
- **P205-025R:** SALSA MLPA Probemix P205 SH2D1A-XIAP-ITK, 25 reactions.
- **P205-050R:** SALSA MLPA Probemix P205 SH2D1A-XIAP-ITK, 50 reactions.
- **P205-100R:** SALSA MLPA Probemix P205 SH2D1A-XIAP-ITK, 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 P205 SH2D1A-XIAP-ITK is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SH2D1A*, *XIAP*, and *ITK* genes, which are associated with Duncan disease.

X-linked lymphoproliferative syndrome (XLP or Duncan disease) is a primary immunodeficiency characterised by severe immune dysregulation, often after viral infection, typically with Epstein-Barr virus (EBV). It is a complex phenotype with symptoms such as severe or fatal mononucleosis, acquired hypogammaglobulinema, hemophagocytic lymphohistiocytosis (HLH), and/or malignant lymphoma. Other features may include aplastic anemia, red cell aplasia, and lymphomatoid granulomatosis. Mutations in the *SH2D1A* (XLP1) and *XIAP* (XLP2) genes on chromosome X are the cause of this disorder. More recently, defects in the IL-2 inducible T cell kinase (*ITK*) gene on chromosome 5 are identified as a cause for an autosomal EBV-associated lymphoproliferative syndrome. It shows a similar, but not identical phenotype to XLP.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1406/.

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 *XIAP*, *SH2D1A*, and *ITK* exon numberings used in this P205-B2 SH2D1A-XIAP-ITK product description are the exon numbering from the RefSeq transcripts NM_001167.3, NM_002351.4, and NM_005546.4, which are identical to the LRG_19, LRG_106, and LRG_189 sequences respectively. As changes to the NCBI database can occur after release of this product description, exon numbering and NM_ sequences used may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P205-B2 SH2D1A-XIAP-ITK contains 39 MLPA probes with amplification products between 129 and 432 nucleotides (nt). This includes eight probes for the *SH2D1A* gene, two probes for all four exons, seven probes for the *XIAP* gene, one probe for each exon, and 16

probes for the *ITK* gene, one probe for each exon with the exception of exon 17. In addition, eight 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.mlpa.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.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)
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.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 Duncan disease. 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 when **reference samples of the same sex** have been used:

Copy number status		Dosage quotient
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < DQ < 1.20
Homozygous deletion	Deletion	DQ = 0
Heterozygous deletion		0.40 < DQ < 0.65
Heterozygous duplication		1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	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 *SH2D1A*, *XIAP*, and *ITK* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P205 SH2D1A-XIAP-ITK.
- 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.

***SH2D1A*, *XIAP*, and *ITK* mutation database:** https://databases.lovd.nl/shared/genes. 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 *XIAP* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P205-B2 SH2D1A-XIAP-ITK

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*SH2D1A*	*XIAP*	*ITK*
64-105	Control fragments – see table in probemix content section for more information				
129 *	Reference probe 19616-L26684	4p13			
137	**ITK probe** 17667-L22567				**Exon 5**
142	**SH2D1A probe** 06105-L20173		**Exon 1**		
148	**ITK probe** 17668-L21750				**Exon 11**
154	**ITK probe** 17669-L21751				**Exon 15**
160	**SH2D1A probe** 06110-L20285		**Exon 4**		
167	**SH2D1A probe** 06106-L05561		**Exon 2**		
172	**SH2D1A probe** 06109-L20174		**Exon 3**		
179	**XIAP probe** 17670-L22564			**Exon 6**	
185	Reference probe 10666-L11248	6p12			
190	**SH2D1A probe** 06104-L05559		**Exon 1**		
196	**SH2D1A probe** 06111-L05566		**Exon 4**		
202	**ITK probe** 17671-L22566				**Exon 4**
211	Reference probe 11015-L11684	15q14			
220	**SH2D1A probe** 06107-L05562		**Exon 2**		
229	**SH2D1A probe** 06108-L05563		**Exon 3**		
238	**XIAP probe** 17673-L21755			**Exon 1**	
244	**ITK probe** 17674-L21756				**Exon 2**
250	**ITK probe** 17675-L21757				**Exon 12**
258	Reference probe 16914-L20514	20q13			
265	**ITK probe** 17676-L21758				**Exon 14**
274 *	Reference probe 21324-L29730	7q31			
283	**ITK probe** 17677-L21759				**Exon 6**
293 Ж	**XIAP probe** 17678-SP0536-L21760			**Exon 7**	
301	**ITK probe** 18311-L21772				**Exon 3**
311	**ITK probe** 17679-L21761				**Exon 7**
319	Reference probe 15385-L17792	3p22			
328	**ITK probe** 17680-L21762				**Exon 9**
337 Ж	**XIAP probe** 17681-SP0537-L22206			**Exon 2**	
346	**ITK probe** 17682-L21764				**Exon 10**
355	**XIAP probe** 17683-L21765			**Exon 5**	
364	**ITK probe** 17684-L21766				**Exon 13**
373	**ITK probe** 17685-L21767				**Exon 1**
382 Ж	**XIAP probe** 17686-SP0538-L21768			**Exon 3**	
391	Reference probe 12928-L14079	2p23			
400	**ITK probe** 17687-L21769				**Exon 16**
409	**XIAP probe** 17688-L21770			**Exon 4**	
417	**ITK probe** 17689-L21771				**Exon 8**
432	Reference probe 14427-L16132	12q21			

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

* New in version B2.
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.

Table 2. P205-B2 probes arranged according to chromosomal location

Table 2a. *XIAP* and *SH2D1A*

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		XIAP	**NM_001167.3**		
		start codon	*160-162 (Exon 2)*		
238	17673-L21755	Exon 1	126-127	CTGTCCTGGCGC-GGTGGGTACAGC	26.2 kb
337 Ж	17681-SP0537-L22206	Exon 2	967-968 and 1004-1005	AAGCACGGATCT-37 nt spanning oligo-GCAGCTTGCAAG	2.1 kb
382 Ж	17686-SP0538-L21768	Exon 3	1039-1040 and 1080-1081	TTTTCGTAGGTG-41 nt spanning oligo-ACTGATTGGAAG	2.5 kb
409	17688-L21770	Exon 4	132 nt before exon 4	TGGGTAACATTT-TACTTTGTGGCT	1.5 kb
355	17683-L21765	Exon 5	145 nt before exon 5 reverse	GGCATAGCCTGA-TGGAGCTAGAAG	8.1 kb
179	17670-L22564	Exon 6	7 nt after exon 6	GAAAGGTATGCA-TTGCTGTTTTTA	6.0 kb
293 Ж	17678-SP0536-L21760	Exon 7	250 nt and 204 nt before exon 7	ATCTTGTCCCAG-46 nt spanning oligo-TGTGCTTAACCT	439.7 kb
		stop codon	*1651-1653 (Exon 7)*		
		SH2D1A	**NM_002351.4**		
		start codon	*362-364 (Exon 1)*		
190	06104-L05559	Exon 1	178-179	TGAGCTCGTTTT-AACTGAAGTGTG	0.1 kb
142	06105-L20173	Exon 1	281-282	AGTCAGGTGGTT-GACTTGTGCCTG	19.0 kb
167	06106-L05561	Exon 2	164 nt before exon 2	GGCAGATACAAT-ATGGAGACAAGT	0.2 kb
220	06107-L05562	Exon 2	10 nt after exon 2	AGGTATAGTTGT-ATTTATTTTTGC	4.1 kb
229	06108-L05563	Exon 3	247 nt before exon 3	GGCAATGAGCAA-GAGCTTGTACAA	0.4 kb
172	06109-L20174	Exon 3	691-692	TCAGCTAGAAGT-ACACAAGGTACT	1.2 kb
160	06110-L20285	Exon 4	850-851	AAATGCATTTCT-AAAGCCATTGTA	1.1 kb
196	06111-L05566	Exon 4	1974-1975	AGATCTGGTCTT-TAGAGGCAGATA	
		stop codon	*746-748 (Exon 4)*		

Table 2b. *ITK*

Length (nt)	SALSA MLPA probe	*ITK* exon[a]	Ligation site NM_005546.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*139-141 (Exon 1)*		
373	17685-L21767	Exon 1	151-152	TGAACAACTTTA-TCCTCCTGGAAG	28.0 kb
244	17674-L21756	Exon 2	324-325	CGAATCAAATGT-GTTGAGATTGTG	2.4 kb
301	18311-L21772	Exon 3	428-429	TCCAGATCGTGA-GAGCCGGCAGCG	3.0 kb
202	17671-L22566	Exon 4	575-576	CTGTGCCCAATA-TGATCCAACCAA	3.6 kb
137	17667-L22567	Exon 5	629-630	TCCTGAAGACAA-CAGGGTGAGTGA	5.0 kb
283	17677-L21759	Exon 6	639-640	CCCCAGCGACCA-CTTTGGGAACCT	5.4 kb
311	17679-L21761	Exon 7	786-787	CCTTTTAACAGG-CATGAAGGATAT	4.1 kb
417	17689-L21771	Exon 8	853-854	TTGCTTTCAGGT-GGTACAATAAGA	5.8 kb
328	17680-L21762	Exon 9	947-948	TTCCAGGACTGC-AGGAACATACAC	2.0 kb
346	17682-L21764	Exon 10	1070-1071	GGCTGAAAAGTA-TGTGTTCGATTC	1.6 kb
148	17668-L21750	Exon 11	1187-1186 reverse	CGTATCTCAGCC-CTGCTGTAACTG	2.0 kb
250	17675-L21757	Exon 12	1290-1291	TACTGGCTCAAC-AAGGACAAGGTG	0.8 kb
364	17684-L21766	Exon 13	56 nt after exon 13	ACAGTTCTCACT-GAAATGACTGGG	1.2 kb
265	17676-L21758	Exon 14	1600-1599 reverse	TCCCACCAAACA-ATTTCTGGCAGC	0.2 kb
154	17669-L21751	Exon 15	1699-1700	GCACCAAATTCC-CGGTGAAGTGGG	2.9 kb
400	17687-L21769	Exon 16	3 nt before exon 16 reverse	TCAGCACACCTA-CAAGAAATGGTG	
		start codon	*1999-2001 (Exon 17)*		

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.

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Related SALSA MLPA probemixes

P028 FHL Contains probes for the *PRF1*, *STX11*, and *UNC13D* genes, involved in Hemophagocytic lymphohistiocytosis (HLH).

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.

P205 Product history	
Version	*Modification*
B2	Two reference probes have been replaced and one reference probe has been removed.
B1	Probes for most exons of the ITK and XIAP genes are now present. All reference probes have been replaced and QDX2 fragments have been added.
A1	First release.

Implemented changes in the product description

Version B2-01 — 16 October 2019 (02P)
- 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 *ITK* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 12 – 06 April 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new pictures included).
- Exon numbering XIAP gene changed according to NM_001167.3.
- Various minor textual changes.

Version 11 (54) – 15 July 2015
- Figure based on the use of old MLPA buffer (replaced in December 2012) removed.

Version 10 (49)
- Product has been renamed.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and 2, new picture included).
- Various textual changes.

Version 09 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.
- Various textual changes.

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