Product Description
SALSA® MLPA® Probemix P240-A5 BRIP1/CHEK1

To be used with the MLPA General Protocol.

Version A5
As compared to version A4, three reference probes have been replaced. For complete product history see page 7.

Catalogue numbers:
- **P240-025R:** SALSA MLPA Probemix P240 BRIP1/CHEK1, 25 reactions.
- **P240-050R:** SALSA MLPA Probemix P240 BRIP1/CHEK1, 50 reactions.
- **P240-100R:** SALSA MLPA Probemix P240 BRIP1/CHEK1, 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 P240 BRIP1/CHEK1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *BRIP1* and *CHEK1* genes, which are associated with hereditary predisposition to cancer.

The *BRIP1* gene may be a target of germline cancer-inducing mutations and has been shown to be mutated in FA-J (FANCJ) cells (Levran et al. 2005). The protein encoded by the *BRIP1* gene is a member of the RecQ DEAH helicase family that interacts with the BRCT repeats of breast cancer type 1 susceptibility protein (BRCA1). The bound complex is important in normal double-strand break repair function of BRCA1.

CHEK1 plays an essential role in the mammalian DNA damage checkpoint (G2/M DNA damage checkpoint), embryonic development, tumour suppression and is regulated by the *ATR* gene (Liu et al. 2000).

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 *BRIP1* and *CHEK1* exon numbering used in this P240-A5 BRIP1/CHEK1 product description is the exon numbering from the LRG_300 and NG_030049.2 sequences. 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 and 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 P240-A5 BRIP1/CHEK1 contains 44 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 13 probes for the *CHEK1* gene and 20 probes for the *BRIP1* gene. In addition, eleven 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 hereditary predisposition to 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *CHEK1* and *BRIP1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P240 BRIP1/CHEK1.

- 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.

COSMIC and LOVD database

http://cancer.sanger.ac.uk/cosmic and https://databases.lovd.nl/shared/genes. We strongly encourage users to deposit positive results in the Catalogue Of Somatic Mutations In Cancer (COSMIC) and 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 *BRIP1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P240-A5 BRIP1/CHEK1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*BRIP1*	*CHEK1*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 09988-L10447	7q		
136	**CHEK1 probe** 08030-L07811			**Exon 13**
142	**BRIP1 probe** 08574-L08575		**Exon 8**	
148	**BRIP1 probe** 08578-L08579		**Exon 12**	
154 *	Reference probe 10694-L11276	6p		
160	**BRIP1 probe** 08583-L08584		**Exon 17**	
166	**CHEK1 probe** 08020-L07801			**Exon 3**
172	**BRIP1 probe** 08585-L08586		**Exon 19**	
178	**CHEK1 probe** 08019-L07800			**Exon 2**
184	Reference probe 21624-L30240	22q		
190	**BRIP1 probe** 08581-L08582		**Exon 15**	
196	**BRIP1 probe** 08569-L08570		**Exon 3**	
202	Reference probe 10880-L14426	15q		
208	**BRIP1 probe** 08580-L08581		**Exon 14**	
215	**BRIP1 probe** 08570-L08571		**Exon 4**	
220	**CHEK1 probe** 08026-L07807			**Exon 9**
229	**CHEK1 probe** 08023-L07804			**Exon 6**
238	Reference probe 08070-L07851	9p		
247	**CHEK1 probe** 08022-L07803			**Exon 5**
256	**CHEK1 probe** 08028-L07809			**Exon 11**
265	**BRIP1 probe** 08575-L08576		**Exon 9**	
281	**CHEK1 probe** 08025-L07806			**Exon 8**
292	**BRIP1 probe** 20076-L27294		**Exon 7**	
301 *	Reference probe 17452-L21208	12p		
310	**BRIP1 probe** 08568-L08569		**Exon 2**	
319	**CHEK1 probe** 08018-L07799			**Exon 1**
328	**BRIP1 probe** 08576-L08577		**Exon 10**	
337	Reference probe 07639-L07324	8p		
346	**CHEK1 probe** 08024-L07805			**Exon 7**
355	**BRIP1 probe** 08571-L08572		**Exon 5**	
364	**BRIP1 probe** 08584-L08585		**Exon 18**	
373	Reference probe 02560-L02023	3q		
382	**BRIP1 probe** 08572-L08573		**Exon 6**	
391	**BRIP1 probe** 08567-L08568		**Exon 1**	
401 *	Reference probe 14865-L16589	14q		
412	**CHEK1 probe** 19883-L27008			**Exon 12**
418	**BRIP1 probe** 08582-L08583		**Exon 16**	
427	**BRIP1 probe** 08577-L08578		**Exon 11**	
436	Reference probe 13809-L15303	5q		
445	**CHEK1 probe** 08021-L07802			**Exon 4**
454	**BRIP1 probe** 08579-L08580		**Exon 13**	
463	**CHEK1 probe** 08027-L07808			**Exon 10**
474	**BRIP1 probe** 08586-L08587		**Exon 20**	
490	Reference probe 18601-L23958	2q		

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

* New in version A5.

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. P240-A5 probes arranged according to chromosomal location

Table 2a. *BRIP1*

Length (nt)	SALSA MLPA probe	*BRIP1* exon[a]	Ligation site NM_032043.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*276-278 (Exon 2)*		
391	08567-L08568	Exon 1	162-163	AGGGCTCCGCTT-TATTTGCTCTCA	1.9 kb
310	08568-L08569	Exon 2	334-333, reverse	ACGGGTAAGCTT-TATAAGGAAAGT	1.6 kb
196	08569-L08570	Exon 3	405-406	AACATTGTTTGT-TGGAGAGTCCCA	2.7 kb
215	08570-L08571	Exon 4	584-585	ATGAACCAAGGA-ACTTCACGTCAT	7.9 kb
355	08571-L08572	Exon 5	700-701	ATCTGCTAAGAA-ACAGGCATCCAT	2.0 kb
382	08572-L08573	Exon 6	821-822	GAAGTACACAAT-TTGGATGCAAAA	38.5 kb
292	20076-L27294	Exon 7	973-974	ATCGAATACCAT-TAAGAAGGATCA	7.3 kb
142	08574-L08575	Exon 8	1312-1313	CCTGGGGAAGAA-ACTAAAGGCCTG	2.1 kb
265	08575-L08576	Exon 9	1496-1497	GAATCAGCAAGT-TACAGTGTAACA	5.6 kb
328	08576-L08577	Exon 10	1722-1721, reverse	AGTAGCAGTGGT-GATACCCATTTT	9.3 kb
427	08577-L08578	Exon 11	1819-1820	GGAGGCAAGAGA-AGTACCTGTTAT	3.4 kb
148	08578-L08579	Exon 12	1960-1961	GACAAATCAGAT-TGATATTTCAGA	0.6 kb
454	08579-L08580	Exon 13	2156-2155, reverse	AATGTAACACCA-AGTTCTGACGAA	3.8 kb
208	08580-L08581	Exon 14	2265-2266	TCTGTGCTACCT-TCCAGAATACTG	32.0 kb
190	08581-L08582	Exon 15	2441-2442	TTGGTGAAGACA-GTCATTGTAGAA	1.4 kb
418	08582-L08583	Exon 16	2569-2570	TCGTGGTAAAGT-GAGTGAGGGTCT	27.1 kb
160	08583-L08584	Exon 17	2704-2705	ATTGAGAGGTCT-TCTACCTGGCCG	22.5 kb
364	08584-L08585	Exon 18	2807-2808	GCTCTTATTCTA-GTGGATGATCGC	7.6 kb
172	08585-L08586	Exon 19	3106-3107	GATATGTGTCCA-GGAACTACAGTG	1.9 kb
474	08586-L08587	Exon 20	3360-3361	CATCAGAGAATA-GTGCCTCTAGTC	
		stop codon	*4023-4025 (Exon 20)*		

Table 2b. *CHEK1*

Length (nt)	SALSA MLPA probe	*CHEK1* exon[a]	Ligation site NM_001114121.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*898-900 (Exon 2)*		
319	08018-L07799	Exon 1	NM_001274.5; 20 nt after exon 1	TAGAATAGTTGT-TCGTGGTTGAAA	0.2 kb
178	08019-L07800	Exon 2	918-919	CCCTTTGTGGAA-GACTGGGACTTG	1.0 kb
166	08020-L07801	Exon 3	1141-1142	ATATCCAATATT-TATTTCTGGAGT	1.5 kb
445	08021-L07802	Exon 4	1215-1216	CCTGAACCAGAT-GCTCAGAGATTC	0.2 kb
247	08022-L07803	Exon 5	1289-1290	TCACAGGGATAT-TAAACCAGAAAA	3.8 kb
229	08023-L07804	Exon 6	1409-1410	GTGTGGTACTTT-ACCATATGTTGC	2.3 kb
346	08024-L07805	Exon 7	16 nt after exon 7	CTGAATTATCTT-GAGTGAAAGAGT	2.0 kb
281	08025-L07806	Exon 8	1664-1665	AAGAATTACCAT-TCCAGACATCAA	6.4 kb
220	08026-L07807	Exon 9	1767-1768	CCCAGTGGATTT-TCTAAGCACATT	0.4 kb
463	08027-L07808	Exon 10	1961-1962	TCATATGCTTTT-GAATAGTCAGTT	0.3 kb
256	08028-L07809	Exon 11	2036-2037	AATGACACGATT-CTTTACCAAATT	9.2 kb
412	19883-L27008	Exon 12	11 nt before exon 12	TAACCTTATTTT-TGTTGCCTTAGG	1.5 kb
136	08030-L07811	Exon 13	2267-2268	ACACTTCCTGAA-GATTAAAGGGAA	
	No probe	*Exon 14*			
		stop codon	*2326-2328 (Exon 13)*		

[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.

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	Hereditary breast cancer; primary screening *BRCA1*.
P087 BRCA1	Results obtained with P002 can be confirmed with this probemix.
P239 BRCA1 region	Characterization of *BRCA1* deletions/duplications.
P045 BRCA2/CHEK2	Hereditary breast cancer, *BRCA2* and *CHEK2*.
P090 BRCA2	Identical to P045 BRCA2/CHEK2 but does not contain probes for *CHEK2*.
P077 BRCA2	Results obtained with P045/P090 can be confirmed with this probemix.
P190 CHEK2	Breast cancer susceptibility, genes included: *CHEK2*, *ATM*, *BRCA1*, *PTEN* and *TP53*.
P057 FANCD2-PALB2	Mutations in *PALB2* have been linked to a higher risk of breast cancer.
P041/P042 ATM	Mutations in *ATM* have been linked to a higher risk of breast cancer.

References

- Levran O et al. (2005) The BRCA1-interacting helicase BRIP1 is deficient in Fanconi Anemia. *Nat Genet*. 37: 931-933.
- Liu Q et al. (2000). Chk1 is an essential kinase that is regulated by Atr an required for the G2/M DNA damage checkpoint. *Genes Dev*. 14: 1448-1459.
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- 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 P240 BRIP1-CHEK1

- Lepkes L et al. (2021). Performance of In Silico Prediction Tools for the Detection of Germline Copy Number Variations in Cancer Predisposition Genes in 4208 Female Index Patients with Familial Breast and Ovarian Cancer. *Cancers 2021*, 13, 118.
- Sato K et al. (2017). Mutation status of RAD 51C, PALB 2 and BRIP 1 in 100 Japanese familial breast cancer cases without BRCA 1 and BRCA 2 mutations. *Cancer Sci*, 108(11), 2287-2294.
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- Velázquez C et al. (2019). Unraveling the molecular effect of a rare missense mutation in BRIP1 associated with inherited breast cancer. *Mol Carcinog*, 58(1), 156-160.
- Wessel K et al. (2017). 17q23. 2q23. 3 de novo duplication in association with speech and language disorder, learning difficulties, incoordination, m
- otor skill impairment, and behavioral disturbances: a case report. *BMC Med Genet*, 18(1), 1-5.

P240 product history	
Version	*Modification*
A5	Three reference probes have been replaced.
A4	Three reference probes have been replaced and one reference probe has been removed.
A3	Five reference probes have been replaced.
A2	The 88, 96, 100 and 105 nt control fragments (QDX2) have been included.
A1	First release.

Implemented changes in the product description
Version A5-01 – 23 April 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 BRIP1 gene updated according to new version of the NM_ reference sequence Version A4-01 – 15 February 2019 (01P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). Version 10 – 26 August 2015 (55) - Product description adapted to a new lot (lot number added, new picture included). - Various minor textual changes. - Exon numbering of the CHEK1 gene has been changed on page 4 and 5.

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