Instructions for Use

SALSA® MLPA® Probemix P016 VHL

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P016 VHL product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P016 VHL**
Version	C2
Catalogue numbers	P016-025R (25 reactions) P016-050R (50 reactions) P016-100R (100 reactions)
Basic UDI-DI	872021148P0165K
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Storage and Shelf Life

Recommended conditions	-25°C -15°C	

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information: www.mrcholland.com	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, the Netherlands
E-mail	info@mrcholland.com (information & technical questions); order@mrcholland.com (orders)
Phone	+31 888 657 200

Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version C1, the 88 and 96 nt DNA Denaturation fragments have been replaced.

1. Intended Purpose

The SALSA MLPA Probemix P016 VHL is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *VHL* and *BRK1* genes in genomic DNA isolated from human peripheral whole blood specimens. P016 VHL is intended to confirm a potential cause for and clinical diagnosis of Von Hippel-Lindau disease (VHL) type I (deletions in *VHL* only) and VHL type IB (deletions encompassing both *VHL* and *BRK1*). In addition, P016 VHL can be utilized for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P016 VHL should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *VHL* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit, and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment • as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of VHL.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*VHL* and *BRK1*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

Study	Description
Expected values for copy numbers in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the product description can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P016 VHL in 70 samples from healthy individuals with normal copy number and 5 samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested, with the exception of two ambiguous measurements.
Limit of detection	A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using SALSA MLPA Probemix P016 VHL on one sample with known *VHL* and *BRK1* CNVs, and on one sample without any CNVs, and expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction. A study using SALSA MLPA Probemix P016 VHL was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known *VHL* and *BRK1* CNVs and samples from healthy individuals. For most probes, FRs within the expected cut-off range were obtained even in the presence of potential interferents at concentrations shown in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 63/66 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 66/66measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 66/66 measurements

	<table><tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 66/66 measurements</td></tr><tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 66/66 measurements</td></tr></table> * Results are summarised for all probes across both samples tested in triplicate. EDTA had the largest effect on copy number determination, as FRs within an incorrect or ambiguous range were found in one sample. EDTA is present during DNA extraction, and in normal concentrations does not affect FRs samples. Therefore, it is only when EDTA is present in excess that ambiguous results for this probe can be found. Ambiguous results in the presence of excess EDTA would at most lead to a delayed result as the assay may have to be repeated. No false positives or negatives would be obtained. None of the other interfering substances affected the FRs in these samples. To minimise variability across samples, 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 where possible.
Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, five previously genotyped samples were tested using SALSA MLPA Probemix P016 VHL and were found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number variations in *VHL* and *BRK1* over multiple days, and by multiple operators. Results showed a correct call in 327/330 data points, leading to a precision of >99%.
Clinical validity*	*VHL*: large intragenic deletions in the *VHL* gene explain 15-42% of the VHL cases (Decker et al. 2014). *VHL* + *BRK1*: large extended *BRK1* deletions explain 6-33% of the VHL cases with a reduced risk of developing renal cell carcinoma (Cascon et al. 2007, Franke et al. 2009, Krzystolik et al. 2014, McNeill et al. 2009, Tamura et al. 2023, Vikkath et al. 2015). *Based on a 2003-2024 literature review

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
3p26.3	*CNTN6*			184	06307-L05830	¬ +
3p25.3	*FANCD2*			283	02138-L01631	¬
3p25.3	*FANCD2*			310	02140-L01633	¬
3p25.3	*BRK1*	Exon 2	0.6 kb	364	14675-L16327	¤
3p25.3	*BRK1*	Exon 3	15.5 kb	193	14676-L16328	¤
3p25.3	*VHL*	Exon 1	0.1 kb	219	01626-L01211	
3p25.3	*VHL*	Exon 1	0.2 kb	274	01628-L01213	
3p25.3	*VHL*	Exon 1	0.2 kb	373	01158-L13266	
3p25.3	*VHL*	Intron 1 (Exon 1)	3.8 kb	328	13625-L15079	Ø
3p25.3	*VHL*	Intron 1 (Exon 2)	0.4 kb	175	13624-L16363	Ø
3p25.3	*VHL*	Exon 2	0.1 kb	201	02390-L16140	+
3p25.3	*VHL*	Exon 2	3.3 kb	391	13322-L14735	
3p25.3	*VHL*	Exon 3	0.1 kb	418	01161-L00717	
3p25.3	*VHL*	Exon 3	28.0 kb	247	01162-L00718	
3p25.3	*IRAK2*			229	02264-L01750	¬
3p25.3	*GHRL*			301	02266-L01752	¬
3p22.2	*MLH1*			238	00892-L00480	¬
5q	Reference			211	13450-L14905	
7q	Reference			346	01335-L00879	«
7q	Reference			427	00680-L00121	
10q	Reference			382	00973-L00560	
11p	Reference			409	00669-L00373	∫
11q	Reference			355	00547-L00116	
13q	Reference			400	00801-L00639	
15q	Reference			265	02454-L01898	
16p	Reference			166	09890-L10303	
17q	Reference			257	01055-L00628	
20p	Reference			319	05981-L05406	
22q	Reference			337	01082-L00660	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *VHL* and *BRK1* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of probes with a target at the edge of or slightly outside the coding region were changed. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

¬ These probes are flanking probes, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested.

« This probe is 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.

Ø These probes target sequences outside of the known coding region. Copy number alterations of only one of these probes are of unknown clinical significance.

\+ The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.

∫ A duplication of this sequence has been observed in healthy individuals. Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home.

¤ Please note that loss of the *BRK1* gene in combination with loss of (part of) the *VHL* gene can be associated with a reduced risk of renal cell carcinoma as compared to defects in the *VHL* gene only, resulting in its designated phenotype VHL type IB.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. The P016 VHL probemix has been used in a research setting to detect *VHL* deletions in tumour tissue in order to identify loss of heterozygosity (LOH) in VHL patients and to study phenotype-genotype correlation (Banks et al. 2006, Patard et al. 2009, Young et al. 2009). **Importantly, the diagnostic use of P016 VHL with DNA extracted from tumour tissue has not been validated**.

5. LOH is often detected at the *VHL* locus in DNA isolated from VHL-associated tumours. Please note that deletion of one copy of the *VHL* gene, which can be detected by MLPA, is only one of the possible causes of LOH. LOH caused by non-disjunction or somatic recombination does not result in a change in copy number of the *VHL* gene and cannot be detected by MLPA.
6. 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.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. MLPA may not be able to identify deletions of *VHL* and *BRK1* in cases in which mosaicism is considered.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Banks RE et al. (2006). Genetic and epigenetic analysis of von Hippel-Lindau (VHL) gene alterations and relationship with clinical variables in sporadic renal cancer. *Cancer Res.* 66:2000-2011.
2. Cascon A et al. (2007). Loss of the actin regulator HSPC300 results in clear cell renal cell carcinoma protection in Von Hippel-Lindau patients. *Hum Mutat.* 28:613-621.
3. Decker J et al. (2014). Clinical utility gene card for: von Hippel-Lindau (VHL). *Eur J Hum Genet.* 22.
4. Franke G et al. (2009). Alu-Alu recombination underlies the vast majority of large VHL germline deletions: Molecular characterization and genotype–phenotype correlations in VHL patients. *Hum Mutat.* 30:776-786.
5. Krzystolik K et al. (2014). Large deletion causing von Hippel-Lindau disease and hereditary breast cancer syndrome. *Hereditary Cancer in Clinical Practice.* 12:16.
6. McNeill A et al. (2009). Genotype-phenotype correlations in VHL exon deletions. *Am J Med Genet A.* 149A:2147-2151.
7. Patard JJ et al. (2009). Absence of VHL gene alteration and high VEGF expression are associated with tumour aggressiveness and poor survival of renal-cell carcinoma. *Br J Cancer.* 101:1417-1424.
8. Tamura K et al. (2023). Variant spectrum of von Hippel-Lindau disease and its genomic heterogeneity in Japan. *Hum Mol Genet.* 32:2046-2054.
9. Vikkath N et al. (2015). Genotype-phenotype analysis of von Hippel-Lindau syndrome in fifteen Indian families. *Fam Cancer.*
10. Young AC et al. (2009). Analysis of VHL Gene Alterations and their Relationship to Clinical Parameters in Sporadic Conventional Renal Cell Carcinoma. *Clin Cancer Res.* 15:7582-7592.

Implemented changes in the product description

Version C2-06 – 19 June 2025 (03S)
- Product description adapted to a new template.
- Intended purpose updated with the inclusion of the extended *BRK1* deletion and its designated phenotype, VHL type IB.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- SNVs rs115744107, rs185858030, rs3087462, rs143428254, rs5030820 and a SNV at +3 from the ligation site of probe 01161-L00717 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Warnings for target sequences outside of the known coding region added for probes 13625-L15079 and 13624-L16363.
- Warnings for a ligation site >20 nt away from the nearest exon added for probes 06307-L05830 and 02390-L16140.
- Probemix is now IVDR-certified.