# Product Description
# SALSA® MLPA® Probemix P492-A1 POLD1 - POLE

To be used with the MLPA General Protocol.

**Version A1**
For complete product history see page 7.

**Catalogue numbers:**
- **P492-025R:** SALSA MLPA Probemix P492 POLD1 - POLE, 25 reactions.
- **P492-050R:** SALSA MLPA Probemix P492 POLD1 - POLE, 50 reactions.
- **P492-100R:** SALSA MLPA Probemix P492 POLD1 - POLE, 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 P492 POLD1 - POLE is a **research use only (RUO)** assay for the detection of deletions or duplications in the *POLD1* and *POLE* genes, which are associated with susceptibility to colorectal cancer. This probemix can also be used to specifically detect the wild-type sequence of the *POLE* c.1270C>G (p.Leu424Val) point mutation.

Susceptibility to colorectal cancer-10 (OMIM #612591) and susceptibility to colorectal cancer-12 (OMIM #615083) are autosomal dominant disorders conferred by mutations in the *POLD1* and *POLE* genes, respectively. Patients show a predisposition to colorectal cancer, with onset at adult age but usually before the age of 40. Brain tumours and endometrial cancer have also been found in *POLD1* and *POLE* mutation carriers. The human *POLD1* and *POLE* genes encode subunits of the polymerase delta (POLδ) and polymerase epsilon (POLε) enzyme complexes, respectively. POLε is responsible for synthesis of the leading strand during DNA replication and POLδ is its equivalent lagging strand polymerase. Besides synthesis, these enzyme complexes also have proof-reading capacity via mismatch and base excision repair pathways. Mutations in *POLD1* and *POLE* have been suggested to cause tumorigenesis due to decreased fidelity of polymerase proof-reading, leading to an increased mutation rate (Palles et al. 2013). The *POLE* c.1270C>G (p.Leu424Val) point mutation affects a highly conserved residue in the *POLE* proofreading domain and has been identified in different families with early-onset colorectal cancer (PMIDs 23447401, 24509466, 24501277, 25529843, 25124163 and 25370038).

The *POLD1* gene (27 exons) spans ~34 kb of genomic DNA and is located on 19q13.33, about 56 Mb from the p-telomere. The *POLE* gene (49 exons) spans ~64 kb of genomic DNA and is located on 12q24.33, about 132 Mb from the p-telomere (close to the q-telomere).

**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 *POLD1* exon numbering used in this P492-A1 POLD1 - POLE product description is the exon numbering from the LRG_785 sequence. The *POLE* exon numbering used in this P492-A1 POLD1 - POLE product description is the exon numbering from the LRG_789 sequence. 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 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 P492-A1 POLD1 - POLE contains 41 MLPA probes with amplification products between 136 and 454 nucleotides (nt). This includes 25 probes for the *POLD1* gene; one probe for each exon except exons 4 and 7. Furthermore, this probemix contains six probes for the *POLE* gene, of which one probe detects the wildtype sequence at the *POLE* c.1270C>G mutation (p.Leu424Val) (see Table 2; **this probe has not been tested on positive samples!**). In addition, 10 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 colorectal cancer, endometrial cancer or brain tumours. 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. Sample ID numbers NA01535, NA02819 and NA07891 from the Coriell Institute have been tested with this P492-A1 probemix at MRC Holland and can be used as positive control samples to detect a heterozygous deletion (NA01535 and NA02819) and a heterozygous duplication (NA07891) of *POLE*. 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 or in or near the *POLE* gene. 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 *POLD1* and *POLE* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P492 POLD1 - POLE.
- 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.

***POLD1* and *POLE* mutation databases**

https://databases.lovd.nl/shared/genes/POLD1 and https://databases.lovd.nl/shared/genes/POLE. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 *POLD1* exons 9 and 11 but not exon 10) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P492-A1 POLD1 - POLE

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | *POLD1* | *POLE* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 136 | Reference probe 19365-L25758 | 3p | | |
| 141 | **POLD1 probe** 22845-L32212 | | **Exon 21** | |
| 148 | **POLE probe** 22895-L32542 | | | **Exon 10** |
| 154 | **POLE probe** 22900-L32294 | | | **Exon 15** |
| 159 | **POLD1 probe** 22846-L32543 | | **Exon 23** | |
| 166 | **POLD1 probe** 22901-L32300 | | **Exon 2** | |
| 172 | Reference probe 13443-L14898 | 17p | | |
| 178 | **POLD1 probe** 22903-L32302 | | **Exon 22** | |
| 184 | **POLD1 probe** 22904-L32303 | | **Exon 6** | |
| 189 | **POLD1 probe** 22905-L32665 | | **Exon 27** | |
| 194 ∞ | **POLE probe** 22899-L32549 | | | **Exon 13** |
| 202 | Reference probe 18560-L24870 | 8q | | |
| 208 « | **POLE probe** 22898-L32295 | | | **Exon 46** |
| 214 | **POLD1 probe** 22847-L32214 | | **Exon 18** | |
| 220 | **POLE probe** 22896-L32664 | | | **Exon 13** |
| 226 | **POLD1 probe** 22848-L32215 | | **Exon 24** | |
| 232 | Reference probe 10410-L12202 | 9q | | |
| 239 | **POLE probe** 22894-L32292 | | | **Exon 2** |
| 244 | **POLD1 probe** 22849-L32547 | | **Exon 8** | |
| 252 | **POLD1 probe** 22850-L32546 | | **Exon 11** | |
| 258 | **POLD1 probe** 22851-L32545 | | **Exon 26** | |
| 265 | Reference probe 10283-L10795 | 2q | | |
| 274 | **POLD1 probe** 22834-L32201 | | **Exon 14** | |
| 283 | **POLD1 probe** 22835-L32202 | | **Exon 9** | |
| 292 | **POLD1 probe** 22836-L32203 | | **Exon 15** | |
| 301 | **POLD1 probe** 22837-L32204 | | **Exon 20** | |
| 310 | Reference probe 16599-L22183 | 16q | | |
| 319 | **POLD1 probe** 22838-L32205 | | **Exon 12** | |
| 328 | **POLD1 probe** 22839-L32544 | | **Exon 5** | |
| 337 | **POLD1 probe** 22840-L32207 | | **Exon 10** | |
| 346 | **POLD1 probe** 22841-L32208 | | **Exon 3** | |
| 355 | Reference probe 10684-L11266 | 6p | | |
| 364 | **POLD1 probe** 22842-L32209 | | **Exon 25** | |
| 372 | **POLD1 probe** 22843-L32210 | | **Exon 16** | |
| 391 | Reference probe 19098-L24985 | 4q | | |
| 400 | **POLD1 probe** 22908-L32307 | | **Exon 17** | |
| 409 | **POLD1 probe** 22909-L32308 | | **Exon 19** | |
| 418 | **POLD1 probe** 23078-L32573 | | **Exon 13** | |
| 427 | Reference probe 21340-L29746 | 7q | | |
| 436 | **POLD1 probe** 22844-L32211 | | **Exon 1** | |
| 454 | Reference probe 16539-L19029 | 15q | | |

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

∞ Wild type sequence detected. This probe is specific for the wildtype sequence at the recurrent c.1270C>G mutation (p.Leu424Val), which has been reported as a cause for a predisposition to colorectal adenomas and carcinomas (PMIDs 23447401, 24509466, 24501277, 25529843, 25124163 and 25370038). A 50% reduced signal for this probe can either be due to this mutation or to a deletion of the sequence detected by this probe. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method. **This probe has not been tested on positive samples!**

« Probe 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, especially of GC-rich regions.

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. P492-A1 probes arranged according to chromosomal location

Table 2a. *POLD1* gene

| Length (nt) | SALSA MLPA probe | *POLD1* exon[a] | Ligation site NM_001256849.1 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *73-75 (Exon 2)* | | |
| 436 | 22844-L32211 | Exon 1 | 53-54 | TGTGGCGGGAAA-CGCTGTTTGAAG | 14.6 kb |
| 166 | 22901-L32300 | Exon 2 | 187-186 reverse | CTCCATCAGTGC-CAGGTCCTCCTC | 0.4 kb |
| 346 | 22841-L32208 | Exon 3 | 291-292 | GTCCCACCATCA-GCCATAGATCCT | 2.6 kb |
| | No probe | Exon 4 | | | |
| 328 | 22839-L32544 | Exon 5 | 574-573 reverse | GGCCAAGTTCAG-CTCCCGTTGCAG | 0.3 kb |
| 184 | 22904-L32303 | Exon 6 | 820-821 | CCAACGTCGACT-TTGAGATCCGGT | 0.3 kb |
| | No probe | Exon 7 | | | |
| 244 | 22849-L32547 | Exon 8 | 946-947 | CGGACGTGCTGT-GGTCTGACGTGG | 0.6 kb |
| 283 | 22835-L32202 | Exon 9 | 1209-intron | GACCTGCTGCAG-GTAGCTCTCGCT | 0.4 kb |
| 337 | 22840-L32207 | Exon 10 | 1289-1290 | CCTTCCGTACCT-CATCTCTCGGGC | 2.7 kb |
| 252 | 22850-L32546 | Exon 11 | 1379-1380 | GGACTCTTCATT-CCAGTCCAAGCA | 0.2 kb |
| 319 | 22838-L32205 | Exon 12 | 1524-1525 | TTCCTGGGCGAG-CAGAAGGAGGAC | 0.7 kb |
| 418 | 23078-L32573 | Exon 13 | 1750-1751 | TATCCCAGCTGT-TGCGGCAGGTCA | 0.2 kb |
| 274 | 22834-L32201 | Exon 14 | 1801-1802 | TGAAGTCAGAGG-GCGGCGAGGACT | 1.5 kb |
| 292 | 22836-L32203 | Exon 15 | 1915-1916 | TGATGGCCCACA-ACCTGTGTTACA | 0.3 kb |
| 372 | 22843-L32210 | Exon 16 | 1987-1988 | ATCAGTTCATCA-GGACCCCCACCG | 0.5 kb |
| 400 | 22908-L32307 | Exon 17 | 2224-2225 | TGGAGATCTCAC-AGGTGGGCACTC | 3.8 kb |
| 214 | 22847-L32214 | Exon 18 | 2287-2288 | TGGAGTCTAAGT-ACACAGTGGAGA | 0.4 kb |
| 409 | 22909-L32308 | Exon 19 | 2440-2441 | TCCCGTCGCCCA-TCCGGCTGGAGT | 1.0 kb |
| 301 | 22837-L32204 | Exon 20 | 2462-2463 | TCCCTCCCAGGT-CTACTTCCCATA | 0.6 kb |
| 141 | 22845-L32212 | Exon 21 | 2638-2639 | TCTCCTACAGAG-ACCCTGAGGGCG | 0.4 kb |
| 178 | 22903-L32302 | Exon 22 | 2887-2888 | CCTACATGAAGT-CGGAGGTCAGGC | 0.6 kb |
| 159 | 22846-L32543 | Exon 23 | 2912-2913 | GTTCGTGCTGGA-GCACAGCCTGCC | 0.3 kb |
| 226 | 22848-L32215 | Exon 24 | 3101-3102 | CAAACGCCGCAA-CTGCTGCATTGG | 0.4 kb |
| 364 | 22842-L32209 | Exon 25 | 3149-3150 | AGGAGCCGTGTG-TGAGTTCTGCCA | 0.2 kb |
| 258 | 22851-L32545 | Exon 26 | 3259-3260 | AGCGCTGCCAGG-GCAGCCTGCACG | 0.7 kb |
| 189 | 22905-L32665 | Exon 27 | 3356-3357 | CCAGGAGCAGCT-CCTGCGGCGCTT | |
| | | *stop codon* | *3394-3396 (Exon 27)* | | |

Table 2b. *POLE* gene

| Length (nt) | SALSA MLPA probe | *POLE* exon[a] | Ligation site NM_006231.4 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *28-30 (Exon 1)* | | |
| 239 | 22894-L32292 | Exon 2 | 193-194 | GGCTGAAGGAGC-CTGGTGAGAAGA | 5.0 kb |
| 148 | 22895-L32542 | Exon 10 | 956-957 | CATCACCAACAG-GGAGATTGTTTC | 1.8 kb |
| 220 | 22896-L32664 | Exon 13 | 707 nt before exon 13 | TGGTTTGGGCTC-GCACTGTAGATT | 0.8 kb |
| 194 ∞ | 22899-L32549 | Exon 13 | 1297-1298 | GCAGTCATAATC-TCAAGGCGGCCG | 0.9 kb |
| 154 | 22900-L32294 | Exon 15 | 1570-1572 | ACGCCAACATCA-TCTTCCCCAACA | 46.5 kb |
| 208 « | 22898-L32295 | Exon 46 | 6371-6372 | GCTGTCCCTGGA-CACCAACATCAC | |
| | | *stop codon* | *6886-6888 (Exon 49)* | | |

[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. This probe is specific for the wildtype sequence at the recurrent c.1270C>G mutation (p.Leu424Val), which has been reported as a cause for a predisposition to colorectal adenomas and carcinomas (PMIDs 23447401, 24509466, 24501277, 25529843, 25124163 and 25370038). A 50% reduced signal for this probe can either be due to this mutation or to a deletion of the sequence detected by this probe. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method. **This probe has not been tested on positive samples!**

« Probe 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, especially of GC-rich regions.

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


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| **P492 product history** | |
|---|---|
| *Version* | *Modification* |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version A1-03 – 16 September 2021 (04P)<br>- Corrected expected results for the samples mentioned in section 'Positive control DNA samples'.<br>- New references added.<br><br>Version A1-02 – 30 August 2021 (04P)<br>- Corrected distances to next probes in table 2b.<br><br>Version A1-01 – 27 July 2021 (04P)<br>- Not applicable, new document. |

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