Instructions for Use

SALSA® MLPA® Probemix P041-B1 ATM-1 and SALSA® MLPA® Probemix P042-B2 ATM-2

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

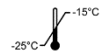
Visit the SALSA® MLPA® Probemix P041 ATM-1 and SALSA® MLPA® Probemix P042 ATM-2 product pages on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P041 ATM-1**
Version	B1
Catalogue numbers	P041-025R (25 reactions) P041-050R (50 reactions) P041-100R (100 reactions)
Basic UDI-DI	872021148P0415J
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Product Name	**SALSA® MLPA® Probemix P042 ATM-2**
Version	B2
Catalogue numbers	P042-025R (25 reactions) P042-050R (50 reactions) P042-100R (100 reactions)
Basic UDI-DI	872021148P0425L
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		

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

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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 P041 Version
As compared to version A3, 12 ATM probes and most reference probes have been replaced. One target probe has been removed. Also, the lengths of some probes have been adjusted.

Changes in P042 Version
As compared to version B1, one target probe has a small change in length.

1. Intended Purpose

The SALSA MLPA Probemix P041 ATM-1 and SALSA MLPA Probemix P042 ATM-2 are in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assays[2] for the detection of deletions or duplications in the *ATM* gene in genomic DNA isolated from human peripheral whole blood specimens. P041 ATM-1 and P042 ATM-2 are intended to confirm a potential cause for and clinical diagnosis of Ataxia-Telangiectasia (in the context biallelic pathogenic variants in ATM) or *ATM*-related cancer susceptibility (in the context of a monoallelic variant in *ATM*), and for molecular genetic testing of at-risk family members. Since only CNVs can be detected with P041 ATM-1 and P042 ATM-2, using an additional method is needed to exclude the possibility of an undetected variant.

In order to cover all *ATM* exons, both P041 ATM-1 and P042 ATM-2 should be used. Copy number variations (CNVs) detected with P041 ATM-1/P042 ATM-2 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 *ATM* gene are point mutations, none of which will be detected by MLPA. It is therefore needed 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, 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, free from heparin, 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 Ataxia-Telangiectasia or *ATM*-related hereditary predisposition to cancer.	
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 (*ATM*)

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 number 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 P041 ATM-1 and SALSA MLPA Probemix P042 ATM-2 in 45 and 44 samples from healthy individuals with normal copy number, respectively, and in five samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested, apart from in one measurement where an ambiguous ratio was obtained. This value would at most lead to delayed results as the assay may need to be repeated. No false positives or false negatives would ensue.
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 P041 ATM-1 and SALSA MLPA Probemix P042 ATM-2 on two samples with known CNVs and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	SNPs 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 P041 ATM-1 and SALSA MLPA Probemix P042 ATM-2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs and a sample with normal copy number. For most probes, expected final ratios (FRs, within the expected cut-off category) 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	P041: Expected FR for 275/297 measurements P042: Expected FR for 280/306 measurements
NaCl	Exogenous – DNA extraction	40 mM	P041: Expected FR for 297/297 measurements P042: Expected FR for 306/306 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	P041: Expected FR for 297/297 measurements

				P042: Expected FR for 305/306 measurements
	Heparin	Exogenous – specimen collection tubes	0.02 U/mL	P041: Expected FR for 297/297 measurements P042: Expected FR for 306/306 measurements
	Hemoglobin	Endogenous – blood sample	0.02 µg/µl	P041: Expected FR for 249/297 measurements P042: Expected FR for 226/306 measurements

* Results are summarised for all ATM probes (P041: 33 probes; P042: 34 probes) across all three samples tested.

Hemoglobin had the largest effect on copy number determination for both P041 ATM-1 and P042 ATM-2: final ratios within an incorrect or ambiguous range were obtained in all samples. DNA extraction methods from blood remove hemoglobin and during testing of 22 samples extracted from blood the expected final ratios were found. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found.

EDTA also had an effect on all samples in both P041 ATM-1 and P042 ATM-2, producing incorrect or ambiguous final ratios. $FeCl_3$ caused only one ambiguous measurement in a *ATM* duplication sample using P042 ATM-2.

Importantly, warnings or errors were obtained in all affected samples (ambiguous or false results) using Coffalyser.Net software.

For the values that fell into the ambiguous range, the worst case scenario would be delayed results, as the assay may have to be repeated. No false positives or false negatives would ensue.

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 when possible.

Cross-reactions	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 P041 ATM-1 and SALSA MLPA Probemix P042 ATM-2 and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 1460/1485 and 1494/1530 data points, respectively, leading to a precision of 98%.
Clinical validity*	Ataxia-Telangiectasia: 5-10% of A-T is caused by large deletions and/or duplications in *ATM* (Gene Reviews). *ATM*-related cancer susceptibility syndrome: around 0.2% of cancer cases are attributable to large deletions or duplications in *ATM* (Hall et al. 2021). *(Based on a 2008-2024 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.

P041 Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
11q22.3	*ATM*	Upstream (Exon 1)	0.1 kb	418	19725-L27158	Ø
11q22.3	*ATM*	Exon 1	1.8 kb	362	19721-L26493	
11q22.3	*ATM*	Intron 1	2.8 kb	173	19806-L27072	Ø
11q22.3	*ATM*	Exon 2	1.6 kb	196	02648-L04595	
11q22.3	*ATM*	Exon 4	6.5 kb	234	02657-L02124	
11q22.3	*ATM*	Exon 5	8.2 kb	280	02660-L26996	
11q22.3	*ATM*	Exon 6	3.0 kb	319	02662-L02129	
11q22.3	*ATM*	Exon 8	3.9 kb	382	19723-L26495	
11q22.3	*ATM*	Exon 10	5.4 kb	136	19701-L26473	
11q22.3	*ATM*	Exon 14	2.7 kb	242	19714-L27329	
11q22.3	*ATM*	Exon 16	8.2 kb	205	02654-L27331	
11q22.3	*ATM*	Exon 17	3.8 kb	142	19703-L26475	
11q22.3	*ATM*	Exon 19	1.7 kb	436	19726-L26498	
11q22.3	*ATM*	Exon 22	6.7 kb	328	02663-L02130	
11q22.3	*ATM*	Exon 23	4.8 kb	373	02667-L04984	
11q22.3	*ATM*	Exon 26	3.4 kb	167	19708-L27071	
11q22.3	*ATM*	Exon 27	2.1 kb	223	19712-L26484	
11q22.3	*ATM*	Exon 29	3.6 kb	310	19720-L26492	
11q22.3	*ATM*	Exon 31	3.9 kb	178	02643-L02110	
11q22.3	*ATM*	Exon 33	2.4 kb	192	03416-L26741	
11q22.3	*ATM*	Exon 34	5.1 kb	255	02658-L26745	
11q22.3	*ATM*	Exon 37	3.1 kb	292	02647-L02114	
11q22.3	*ATM*	Exon 38	4.5 kb	337	02664-L02131	
11q22.3	*ATM*	Exon 40	3.7 kb	274	19719-L26491	
11q22.3	*ATM*	Exon 42	3.9 kb	427	02671-L27157	
11q22.3	*ATM*	Exon 44	5.4 kb	463	02674-L02141	
11q22.3	*ATM*	Exon 46	3.8 kb	148	03414-L03275	
11q22.3	*ATM*	Exon 49	3.6 kb	184	02653-L02120	
11q22.3	*ATM*	Exon 53	3.1 kb	217	02656-L02123	
11q22.3	*ATM*	Exon 56	7.4 kb	261	02659-L26744	
11q22.3	*ATM*	Exon 57	4.1 kb	287	03418-L26743	
11q22.3	*ATM*	Exon 59	7.5 kb	345	19809-L02132	
11q22.3	*ATM*	Exon 61	10.2 kb	391	02642-L02109	
11q22.3	*ATM*	Exon 62		249	19715-L26995	
2q	Reference			154	13816-L15310	
3q	Reference			130	16316-L18705	
5p	Reference			268	03075-L19995	
7q	Reference			160	07992-L07773	
8q	Reference			355	01045-L00615	
13q	Reference			445	16286-L18578	
15q	Reference			229	15079-L26739	
16q	Reference			301	04570-L23473	
17q	Reference			409	07455-L07103	
18q	Reference			485	16456-L18909	
20q	Reference			209	16261-L27376	

P042 Content – Probe Details Sorted by Chromosomal Position

Chr.position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
11q22.3	*ATM*	Exon 3	17.1 kb	150	08415-L26617	
11q22.3	*ATM*	Exon 7	4.1 kb	250	19713-L27950	
11q22.3	*ATM*	Exon 9	3.0 kb	463	19728-L26500	
11q22.3	*ATM*	Exon 11	0.8 kb	215	19711-L27805	
11q22.3	*ATM*	Exon 12	1.1 kb	412	19802-L26626	
11q22.3	*ATM*	Exon 13	3.6 kb	373	08420-L08326	
11q22.3	*ATM*	Exon 15	11.0 kb	262	19717-L27949	
11q22.3	*ATM*	Exon 18	2.9 kb	292	08422-L26622	
11q22.3	*ATM*	Exon 20	1.2 kb	448	19727-L26499	
11q22.3	*ATM*	Exon 21	8.6 kb	156	19705-L26878	
11q22.3	*ATM*	Exon 24	1.6 kb	191	19803-L26627	
11q22.3	*ATM*	Exon 25	6.4 kb	209	08426-L26619	
11q22.3	*ATM*	Exon 28	3.6 kb	170	S1099-SP0950-L27632	Ж #
11q22.3	*ATM*	Exon 30	2.4 kb	142	19704-L26476	
11q22.3	*ATM*	Exon 32	6.6 kb	161	19706-L26478	
11q22.3	*ATM*	Exon 35	1.3 kb	203	19710-L26882	
11q22.3	*ATM*	Exon 36	7.2 kb	328	08431-L08322	
11q22.3	*ATM*	Exon 39	5.7 kb	382	19722-L26494	
11q22.3	*ATM*	Exon 41	1.6 kb	341	08433-L26623	
11q22.3	*ATM*	Exon 43	3.9 kb	178	19709-L26481	
11q22.3	*ATM*	Exon 45	4.8 kb	311	19808-L27211	
11q22.3	*ATM*	Exon 47	1.5 kb	220	08436-L27803	
11q22.3	*ATM*	Exon 48	2.6 kb	198	08437-L26618	
11q22.3	*ATM*	Exon 50	1.2 kb	358	19350-L08325	
11q22.3	*ATM*	Exon 51	0.5 kb	280	08439-L27947	
11q22.3	*ATM*	Exon 52	2.0 kb	346	09367-L26624	
11q22.3	*ATM*	Exon 54	1.1 kb	394	09667-L26625	
11q22.3	*ATM*	Exon 55	10.9 kb	243	08442-L27951	
11q22.3	*ATM*	Exon 58	8.0 kb	427	08443-L08330	
11q22.3	*ATM*	Exon 60	1.6 kb	232	08444-L27210	
11q22.3	*ATM*	Intron 61 (Exon 61)	7.9 kb	273	19718-L27948	Ø
11q22.3	*ATM*	Intron 61	0.7 kb	474	19729-L26501	Ø
11q22.3	*ATM*	Intron 61	1.4 kb	419	19724-L26496	Ø
11q22.3	*ATM*	Exon 63		286	08445-L26621	
1q	Reference			436	14775-L16472	
3q	Reference			319	12552-L13602	
3q	Reference			131	16316-L22397	
4q	Reference			256	10808-L27953	
5q	Reference			365	14059-L26885	
6q	Reference			301	14941-L16674	
7p	Reference			136	07905-L27802	
8q	Reference			401	10638-L12897	
9p	Reference			184	06658-L06231	
13q	Reference			454	02144-L01619	
18q	Reference			485	16456-L18909	

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 *ATM* exon numbers are derived from MANE project and are based on MANE Select. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with targets at the edge of or slightly outside the coding region, were altered. The exon numbering from the previous version of this Product Description is disclosed between brackets when a discrepancy is present.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

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.

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

\# The specificity of this probe relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Deletions of the last *ATM* exons (exon 62-63) are encountered frequently (own validation observations, Micol et al. 2011, Nakamura et al. 2012, Podralska et al. 2014, Susswein et al. 2016, Tung et al. 2015). Duplication of these exons might not result in inactivation of that gene copy and should therefore be interpreted with caution (Lepkes et al. 2021, Nurmi et al. 2022).
6. For the P041 ATM-1 probemix, we observed a prominent peak with a length of approximately 480 nt in a no-DNA control. This peak has a height <25% of the median of the four Q-fragments and is therefore not expected to affect MLPA reactions when sufficient (50-250 ng) sample DNA is used. Deviations from the protocol may increase the height of this peak in reactions with and without DNA.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. 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 (Basel)*. 13.
2. Micol R et al. (2011). Morbidity and mortality from ataxia-telangiectasia are associated with ATM genotype. Journal of Allergy and Clinical Immunology. 128:382-389. e381.
3. Nakamura K et al. (2012). Functional characterization and targeted correction of ATM mutations identified in Japanese patients with ataxia-telangiectasia. Hum Mutat. 33:198-208.
4. Nurmi AK et al. (2022). Pathogenic Variant Spectrum in Breast Cancer Risk Genes in Finnish Patients. *Cancers*. 14:6158.
5. Podralska MJ et al. (2014). Ten new ATM alterations in Polish patients with ataxia-telangiectasia. Mol Genet Genomic Med. 2:504-511.
6. Susswein LR et al. (2016). Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. Genet Med. 18:823-832.
7. Tung N et al. (2015). Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 121:25-33.

Implemented changes in the product description

Version B1-B2-04 – 20 June 2025 (03S)
- Product description updated to new template.
- Intended purpose was modified by making distinctions between Ataxia-Telangiectasia and ATM-related cancer susceptibility. It is also now specified that an additional method is needed to exclude the possibility of an undetected variant.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warning for target outside the transcript region added for probes 19725-L27158, 19806-L27072, 19718-L27948, 19729-L26501, and 19724-L26496.
- SNVs rs587780628 and rs201159454 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.
- Performance characteristics updated with data from analytical performance experiments.
- Probemixes are no longer registered for IVD use in Morocco.
- Probemixes are now IVDR-certified.