Instructions for Use

SALSA® MLPA® Probemix P015 MECP2

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 P015 MECP2 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P015 MECP2**
Version	F2
Catalogue numbers	P015-025R (25 reactions) P015-050R (50 reactions) P015-100R (100 reactions)
Basic UDI-DI:	872021148P0155H
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 COLOMBIA ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information: **www.mrcholland.com**	
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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 in which the user and/or the patient is located.

Changes in this Product Version:
As compared to version F1, two reference probes have been replaced and the 118 nt Y fragment has been removed.

1. Intended Purpose

The SALSA MLPA Probemix P015 MECP2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *MECP2* gene, in order to confirm a potential cause for or clinical diagnosis of Rett syndrome (deletions in *MECP2*) and *MECP2* duplication syndrome (duplications in *MECP2*). P015 MECP2 can also be used for the detection of deletions in the *CDKL5* gene, in order to confirm a potential cause for or clinical diagnosis of CDKL5 deficiency disorder. This assay is additionally intended for molecular genetic testing of at-risk family members, concerning *MECP2* duplication syndrome, and is intended for use with genomic DNA isolated from human peripheral whole blood specimens[3].

Copy number variations (CNVs) detected with P015 MECP2 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 *MECP2* and *CDKL5* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Not all exons of the *CDKL5* gene are covered. The SALSA MLPA Probemix P189 CDKL5/ARX/FOXG1 is available for the detection of deletions in each exon of *CDKL5*.

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.

[1] Please note that this probemix is for IVD use in the countries specified at the end 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.

[3] Certain probes targeting additional genes included in P015 MECP2 may only be used in a research setting. The following table summarises which probes are for IVD use or exclusively restricted to be used in a research setting:

IVD Targets	RUO Targets
MECP2, CDKL5	*NTNG1, ARX*

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 Rett syndrome (classic and atypical), CDKL5 deficiency disorder, or DEE1. • Need to be of the same sex (all male, or all female) for correct data analysis, and it is recommended to use reference samples of the same sex as patient samples, for ease of interpretation.	
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.
Validation samples (required)	• In the validation experiments of this probemix, DNA samples from healthy individuals of the same sex should be used.	

*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 X Probes (Compared to Same Gender)

Final Ratio (FR)	Copy Number Female	Copy Number Male	Description
0	0	0	**Female:** Homozygous deletion **Male**: Deletion
0.40 – 0.65	1	-	**Female:** Heterozygous deletion
0.80 – 1.20	**2**	**1**	**Normal**
1.30 – 1.65	3	-	**Female:** Heterozygous duplication
1.75 – 2.15	4	2	**Female**: Homozygous duplication or Heterozygous triplication **Male:** Duplication
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 above can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P015 MECP2 in 45 samples from healthy individuals with normal copy numbers and 10 samples with known CNVs. The expected FRs for the corresponding copy number were found in 99% of measurements over all samples tested. Ambiguous FRs were obtained in the remaining 1%.
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 P015 MECP2 on three samples with known CNVs and on one normal sample and expected results were obtained in 98% of cases overall, using both the lower and upper input amount of DNA. Ambiguous FRs were obtained for the remaining 2%.
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 P015 MECP2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs and one normal sample. For most probes, FRs within the expected cut-off category were obtained even in the presence of potential interferents at concentrations shown in the table below.

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td></td><td>
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 188/216 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 175/216 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 208/216 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 209/216 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 144/216 measurements</td></tr>
</table>
* Results are summarised for all probes across all four samples tested.

Hemoglobin had the largest effect on copy number determination: final ratios within an incorrect or ambiguous range were obtained in all samples. DNA extraction methods from blood remove hemoglobin. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found.

NaCl also had a strong effect on most samples, yielding either incorrect or ambiguous results. This is not unexpected, as several probes in the mix are known to be salt sensitive. There are warnings present for these probes in the product description.

EDTA had an effect on two samples, producing ambiguous results for several probes. Such values would at most lead to delayed results as the assay may have to be repeated. No false positives or false negative would ensue.

Additionally, Coffalyser.Net issues warnings for the samples in which the interferents showed an effect, as well as lowered quality scores, this also leading to the samples needing a re-test according to the IFU.

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.
</td></tr>
<tr><td>Cross-reactivity</td><td>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.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, ten previously genotyped samples were tested using SALSA MLPA Probemix P015 MECP2 and found to have the expected results in 99% of cases.

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 896/960 data points, leading to a precision of 93%.</td></tr>
<tr><td>Clinical validity*</td><td>*MECP2*: large deletions in *MECP2* explain 5-10% of the RTT cases (Archer et al. 2006; Hardwick et al. 2007; Pan et al. 2006; Philippe et al. 2006; Zahorakova et al. 2007), and duplications of *MECP2* explain 100% of the *MECP2* duplication syndrome cases[1].

CDKL5: approximately 6.5-10% of the patients with CDD have large deletions in *CDKL5*[2].

*Based on a 2003-2024 literature review</td></tr>
</table>

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.

[1] Gene Reviews – MECP2 duplication syndrome
https://www.ncbi.nlm.nih.gov/books/NBK1284/#:~:text=MECP2%20duplication%20syndrome%20should%20be,predominantly%20of%20the%20lower%20limbs
[2] RettBASE http://mecp2.chw.edu.au/

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
1p13.3	*NTNG1*	Exon 2	176.5 kb	149	18447-L24143	
1p13.3	*NTNG1*	Exon 3	83.1 kb	438	06483-L24162	
1p13.3	*NTNG1*	Intron 5 (Exon 5)	72.7 kb	312	06487-L24154	Ø
1p13.3	*NTNG1*	Exon 8 (6)		427	06488-L23934	
Xp22.13	*CDKL5*	Exon 3	69.1 kb	190	06458-L23617	+
Xp22.13	*CDKL5*	Exon 6	8.2 kb	286	06462-L24150	
Xp22.13	*CDKL5*	Exon 9	7.3 kb	400	06465-L05991	
Xp22.13	*CDKL5*	Exon 10	6.4 **M**b	144	06466-L06567	
Xp21.3	*ARX*	Exon 5	11.2 kb	235	06455-L21229	«
Xp21.3	*ARX*	Exon 1	127.7 **M**b	161	18440-L24338	« ∫
Xq28	*SLC6A8*		99.8 kb	172	01879-L24147	¬ « # ∫
Xq28	*IDH3G*		70.3 kb	384	01887-L23933	¬
Xq28	*L1CAM*		154.4 kb	241	07051-L14553	¬
Xq28	*IRAK1*		5.9 kb	178	10835-L24148	¬ «
Xq28	*MECP2*	Exon 3 (4)	3.2 kb	260	01769-L23834	«
Xq28	*MECP2*	Exon 3 (4)	2.2 kb	418	18446-L23620	«
Xq28	*MECP2*	Exon 3 (4)	0.1 kb	292	18444-L24151	
Xq28	*MECP2*	Exon 3 (4)	0.1 kb	274	01768-L13824	
Xq28	*MECP2*	Exon 3 (4)	0.2 kb	155	18442-L24243	¤
Xq28	*MECP2*	Exon 3 (4)	0.3 kb	346	01347-L24157	¤ «
Xq28	*MECP2*	Exon 3 (4)	0.3 kb	229	18441-L12494	
Xq28	*MECP2*	Exon 3 (4)	0.7 kb	356	10841-L24158	+
Xq28	*MECP2*	Exon 2 (3)	0.2 kb	391	14737-L24161	«
Xq28	*MECP2*	Exon 2 (3)	0.2 kb	365	01348-L24159	
Xq28	*MECP2*	Exon 2 (3)	59.6 kb	196	10839-L23618	+
Xq28	*MECP2*	Intron 1 (Exon 2)	0.1 kb	373	01349-L24160	« Ø ±
Xq28	*MECP2*	Intron 1 (Exon 2)	5.3 kb	254	03768-L23833	« Ø ±
Xq28	*MECP2*	Exon 1	0.4 kb	184	10836-L24339	«
Xq28	*MECP2*	Upstream (Exon 1)	0.1 kb	202	03409-L16570	« Ø
Xq28	*MECP2*	Upstream (Exon 1)	0.7 kb	305	02002-L24153	« Ø
Xq28	*MECP2*	Upstream	221.7 kb	214	03770-L13387	« Ø
Xq28	*FLNA*		79.7 kb	447	04138-L24163	¬ «
Xq28	*GDI1*		331.9 kb	137	16875-L19669	¬ « ∫
Xq28	*DKC1*		218.1 kb	319	18761-L24340	¬
Xq28	*F8*		957.0 kb	221	06288-L05892	¬
Xq28	*VAMP7*			457	01094-L24164	¬
1q	Reference			299	16621-L24152	
5q	Reference			130	00797-L21056	
6p	Reference			266	10728-L22588	
7p	Reference			247	05959-L05376	
10q	Reference			166	08222-L24146	
11q	Reference			467	02674-L24165	
12p	Reference			409	17462-L21218	
13q	Reference			208	09865-L08705	
15q	Reference			338	09776-L24156	
18q	Reference			280	16434-L24149	

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 *NTNG1, MECP2, ARX* and *CDKL5* 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 several one 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.
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.

« These probes are 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 the known coding region. Copy number alterations of only 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.

¤ Probe located immediately adjacent to the deletion-prone region (DPR) in *MECP2*, in which copy number changes and SNVs occur frequently (Huppke and Gartner 2005, Laccone et al. 2004, Vidal et al. 2019).

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

∫ The presence of salt in DNA samples can result in incomplete denaturation of CpG islands, which may result in false positive results: apparent deletions of these probes should be handled with care. Coffalyser.Net issues a sample denaturation warning when the signal intensity of the 88 nt and/or 96 nt D-fragments are too low. These probes target extremely GC-rich chromosomal areas, and are affected by salt concentrations that not yet affect the control D-fragments, thus without Coffalyser.Net issuing a warning. False positive results are more likely when DNA has been extracted by the Qiagen EZ1, M48 or M96 systems, as these leave a higher salt concentration in the sample. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration or other related technique).

± These probes target coding sequences in alternative transcripts.

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. Deletion of a probe's recognition sequence on the X-chromosome will lead to a complete absence of the corresponding probe amplification product in males, whereas female heterozygotes are recognizable by a 35-50% reduction in relative peak height.
6. The use of fixed cut-off values for the FR of the probes may not allow detection of mosaic deletions or duplications. Mosaic *CDKL5* deletions have been reported in CDKL5 deficiency disorder (Bartnik et al. 2011, Boutry-Kryza et al. 2014, Mei et al. 2014). In order to detect mosaic samples, the experiment has to have little variation and the final ratios should be significantly different from the reference samples (see Coffalyser.Net Reference Manual, Appendix – Normalisation and result interpretation). Mosaic samples may not be detected if the percentage of cells that have the deletion or duplication is low.

Technique-specific precautions

See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. Target probes for *NTNG1* and *ARX* CNVs are included to be used for research purposes only and not for diagnostic use.

Technique-specific limitations

See the MLPA General Protocol.

9. References Cited in this IFU

1. Archer, H.L., et al., Gross rearrangements of the MECP2 gene are found in both classical and atypical Rett syndrome patients. J Med Genet, 2006. 43(5): p. 451-6.
2. Hardwick, S.A., et al., Delineation of large deletions of the MECP2 gene in Rett syndrome patients, including a familial case with a male proband. Eur J Hum Genet, 2007. 15(12): p. 1218-29.
3. Pan, H., et al., Large deletions of the MECP2 gene in Chinese patients with classical Rett syndrome. Clin Genet, 2006. 70(5): p. 418-9.
4. Philippe, C., et al., Spectrum and distribution of MECP2 mutations in 424 Rett syndrome patients: a molecular update. Eur J Med Genet, 2006. 49(1): p. 9-18.
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6. Shoubridge, C., T. Fullston, and J. Gecz, ARX spectrum disorders: making inroads into the molecular pathology. Hum Mutat, 2010. 31(8): p. 889-900.
7. Borg, I., et al., Disruption of Netrin G1 by a balanced chromosome translocation in a girl with Rett syndrome. Eur J Hum Genet, 2005. 13(8): p. 921-7.
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9. Huppke, P. and J. Gartner, Molecular diagnosis of Rett syndrome. J Child Neurol, 2005. 20(9): p. 732-6.
10. Vidal, S., et al., Characterization of large deletions of the MECP2 gene in Rett syndrome patients by gene dosage analysis. Mol Genet Genomic Med, 2019. 7(8): p. e793.
11. Bartnik, M., et al., Early-onset seizures due to mosaic exonic deletions of CDKL5 in a male and two females. Genet Med, 2011. 13(5): p. 447-52.
12. Boutry-Kryza, N., et al., Complex mosaic CDKL5 deletion with two distinct mutant alleles in a 4-year-old girl. Am J Med Genet A, 2014. 164A(8): p. 2025-8.
13. Mei, D., et al., Optimizing the molecular diagnosis of CDKL5 gene-related epileptic encephalopathy in boys. Epilepsia, 2014. 55(11): p. 1748-53.

Implemented changes in the product description
Version F2-08 – 26 March 2025 (03S) - Product Description updated to new template. - Product now IVD in Colombia. - Intended purpose updated by removal of the *NTNG1* and *ARX* genes, and early infantile epileptic encephalopathy 1 (EIEE1). Genetic testing of at-risk family members is now confined to MECP2 duplication syndrome. CNV type detected in the *CDKL5* gene limited to deletions. - Specification regarding the use of NTNG1 and ARX probes added in section Limitations. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Exon numbering for several NTNG1 and MECP2 probes was updated . - Warning for probes with ligation site >20nt away from the nearest exon added for probes 10839-L23618, 10841-L24158, and 06458-L23617. - SNV rs267608346 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes, included in section Precautions and Warnings. - Warning for the target being outside the transcript region added for probes 06487-L24154, 03770-L13387, 02002-L24153, 03409-L16570, 03768-L23833, and 01349-L24160. - Warning for salt sensitivity added for probes 01347-L24157 and 14737-L2416. - Performance Characteristics section updated with data from analytical performance experiments. - Probemix is now IVDR certified.