Instructions for Use

SALSA® MLPA® Probemix P189 CDKL5-FOXG1

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 P189 CDKL5-FOXG1 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P189 CDKL5-FOXG1**
Version	**C2**
Catalogue numbers	**P189-025R (25 reactions)** **P189-050R (50 reactions)** **P189-100R (100 reactions)**
Basic UDI-DI	**872021148P1896K**
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

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 C1, several probes have been adjusted in length, but no change in sequence detected. The name of the probemix has been changed from "CDKL5/ Atypical Rett syndrome" to "CDKL5/ARX/FOXG1", then to "CDKL5-FOXG1" (2026).

1. Intended Purpose

The SALSA MLPA Probemix P189 CDKL5-FOXG1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *CDKL5* gene, in order to confirm a potential cause for or clinical diagnosis of CDKL5 deficiency disorder. P189 CDKL5-FOXG1 can also be used for the detection of deletions or duplications in the *FOXG1* gene, in order to confirm a potential cause for or clinical diagnosis of *FOXG1* syndrome. This assay is intended for use with genomic DNA isolated from human peripheral whole blood specimens[3].

Copy number variations (CNVs) detected with P189 CDKL5-FOXG1 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 *CDKL5* and *FOXG1* 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.

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 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.

[3]Certain probes targeting additional genes included in P189 CDKL5-FOXG1 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
CDKL5, FOXG1	*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 CDKL5 deficiency disorder or FOXG1 syndrome. • 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 Probes Targeting Two Copies (*FOXG1*)

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

Typical Results of X Probes (Compared to Same Sex)

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 P189 CDKL5-FOXG1 in 44 samples from healthy individuals with normal copy numbers and eight samples with known CNVs. The expected FRs for the corresponding copy number were found in 99% of all samples tested. In the remaining 1% of cases, ambiguous values were obtained.
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 P189 CDKL5-FOXG1 on three samples with known CNVs and on one sample without any mutation and expected results were obtained in all cases 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 P189 CDKL5-FOXG1 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, expected FRs (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	Copy number: Expected FR for 252/264 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 261/264 measurements

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td></td><td>
<table>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 264/264 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 264/264 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 209/264 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 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.

EDTA had a milder effect on probe signals, leading to ambiguous FRs in one sample, and both ambiguous and incorrect values in another sample. Ambiguous values would at most lead to delayed results, as the assay maybe have to be repeated. No false positives or false negatives would ensue. In one of these samples, the false results were obtained only for one CDKL5 exon 1 probe over all three replicates. There are two probes covering this exon, thus a discrepancy between the obtained FRs should be investigated using a follow-up method by a professional in the field, as is stated in the product description.

NaCl only had an effect on the FOXG1 probe of the wildtype sample (in all three replicates). A warning for this probe is also present in the Product Description.

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 P189 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-reactions</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, eight previously genotyped samples were tested using SALSA MLPA Probemix P189 CDKL5-FOXG1 and found to have the expected results in 97% 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 1314/1320 data points, leading to a precision of >99%.</td></tr>
<tr><td>Clinical validity*</td><td>*CDKL5*: approximately 6.5-10% of the patients with CDD have large deletions in *CDKL5*[1].

FOXG1: the percentage of *FOXG1* syndrome cases explained by large deletions or duplications in *FOXG1* varies depending on the phenotype examined, but has been estimated at ~11% (Vegas et al. 2018).

*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] 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*	Upstream (Exon 1)	0.1 kb	149	06478-L06568	Ø
1p13.3	*NTNG1*	Upstream (Exon 1)	7.8 kb	202	06479-L06005	Ø
1p13.3	*NTNG1*	Exon 2	0.5 kb	257	06480-L21520	
1p13.3	*NTNG1*	Exon 2	175.8 kb	310	06481-L06007	
1p13.3	*NTNG1*	Exon 3	0.4 kb	391	06482-L06008	
1p13.3	*NTNG1*	Exon 3	70.3 kb	436	06483-L06009	
1p13.3	*NTNG1*	Exon 4	0.2 kb	178	06484-L06010	
1p13.3	*NTNG1*	Exon 4	12.1 kb	274	06485-L29843	+
1p13.3	*NTNG1*	Intron 4 (Exon 5)	0.5 kb	208	06486-L06572	Ø
1p13.3	*NTNG1*	Intron 5 (Exon 5)	72.7 kb	305	06487-L29847	Ø
1p13.3	*NTNG1*	Exon 8 (6)	0.7 kb	427	06488-L06014	
1p13.3	*NTNG1*	Exon 8 (6)		364	06489-L06015	
14q12	*FOXG1*	Upstream	2.5 kb	333	16850-L19644	« Ø
14q12	*FOXG1*	Exon 1		472	17292-L15242	«
Xp22.13	*CDKL5*	Exon 1	0.1 kb	154	13667-L15127	
Xp22.13	*CDKL5*	Exon 1	81.2 kb	136	06456-L24567	+
Xp22.13	*CDKL5*	Exon 2	3.8 kb	161	06457-L06570	
Xp22.13	*CDKL5*	Exon 3	53.7 kb	185	06458-L05984	+
Xp22.13	*CDKL5*	Exon 4	0.2 kb	227	06459-L23728	
Xp22.13	*CDKL5*	Exon 4	10.8 kb	452	21264-L05986	+
Xp22.13	*CDKL5*	Exon 5	4.4 kb	246	06461-L23731	
Xp22.13	*CDKL5*	Exon 6	2.0 kb	292	06462-L29846	
Xp22.13	*CDKL5*	Exon 7	2.4 kb	319	06463-L05989	
Xp22.13	*CDKL5*	Exon 8	3.8 kb	373	06464-L05990	
Xp22.13	*CDKL5*	Exon 9	7.3 kb	400	06465-L05991	
Xp22.13	*CDKL5*	Exon 10	3.1 kb	144	06466-L06567	
Xp22.13	*CDKL5*	Exon 11	5.7 kb	172	06467-L06571	
Xp22.13	*CDKL5*	Exon 12	4.6 kb	340	06882-L21233	
Xp22.13	*CDKL5*	Exon 13	0.6 kb	445	06469-L05995	
Xp22.13	*CDKL5*	Exon 14	3.7 kb	252	06470-L16143	
Xp22.13	*CDKL5*	Exon 15	6.7 kb	326	06471-L05997	
Xp22.13	*CDKL5*	Exon 16	4.0 kb	383	06472-L05998	
Xp22.13	*CDKL5*	Intron 16	1.3 kb	286	21263-L29845	Ø
Xp22.13	*CDKL5*	Exon 17	3.3 kb	418	06473-L05999	
Xp22.13	*CDKL5*	Exon 18	17.6 kb	239	06474-L23730	
Xp22.13	*CDKL5*	Downstream (Exon 19)	4.4 kb	463	06475-L06001	Ø
Xp22.13	*CDKL5*	Downstream (Exon 20)	3.2 kb	298	06476-L26217	Ø
Xp22.13	*CDKL5*	Downstream (Exon 21)	6.3 **M**b	481	17286-L20734	Ø
Xp21.3	*ARX*	Exon 5	2.5 kb	265	21262-L29842	«
Xp21.3	*ARX*	Exon 4	2.9 kb	222	02898-L04200	« ∫
Xp21.3	*ARX*	Exon 3	3.0 kb	355	13670-L21234	« ∫
Xp21.3	*ARX*	Exon 2	2.8 kb	196	18790-L24442	« ∫
Xp21.3	*ARX*	Exon 1		233	13669-L23729	« ∫
1q	Reference			280	12494-L29844	
4p	Reference			191	06057-L06042	
5q	Reference			130	00797-L13645	
7p	Reference			166	07724-L07434	
11q	Reference			409	09497-L09754	
15q	Reference			490	09772-L21655	
17q	Reference			215	08570-L08571	
18q	Reference			348	16441-L18894	
20q	Reference			500	17001-L22947	

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 *CDKL5, ARX, NTNG1,* and *FOXG1* 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 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 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 this probe 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

sequence sheet from the probemix-specific page on 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 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).

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 I – 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. Vegas, N., et al., Delineating FOXG1 syndrome: From congenital microcephaly to hyperkinetic encephalopathy. Neurol Genet, 2018. 4(6): p. e281.
2. 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.
3. 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.
4. 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 C2-07 – 27 May 2026 (03S)

- Product name updated from "P189 CDKL5/ARX/FOXG1" to "P189 CDKL5-FOXG1". This change was also implemented in the Intended Purpose.

Version C2-06 – 27 March 2025 (03S)

- Product Description updated to new template.
- Intended Purpose updated by removal of the *NTNG1* and *ARX* genes, and early infantile epileptic encephalopathy 1 (EIEE1) and atypical Rett syndrome. CNV type detected in the *CDKL5* gene limited to deletions. The probemix is no longer intended for use for at-risk family member testing.
- Specification regarding the use of the NTNG1 and ARX probes added to 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.
- Warnings for salt sensitivity removed for probes 13667-L15127, 06456-L24567, 06478-L06568, 06479-L06005, 06480-L21520, and 06481-L06007.
- Exon numbering for several NTNG1 and CDKL5 probes was updated.
- SNVs rs147336854, rs2587905, and rs144330931 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.
- Warning for probes with ligation site >20nt away from the nearest exon added for probes 06456-L24567, 06458-L05984, 21264-L05986, and 06485-L29843.
- Warning for the target being outside the transcript region added for probes 21263-L29845, 06479-L06005, 06486-L06572, 06487-L29847, and 16850-L19644.
- Performance Characteristics section updated with data from analytical performance experiments.
- Probemix is now IVDR certified.