Product Description
SALSA® MLPA® Probemix P395-B1 MEF2C-FOXG1

To be used with the MLPA General Protocol.

Version B1
As compared to version A2, one target probe has been replaced, two target probes have been added, six reference probes have been replaced, and four target probes have been changed in length, not in sequence detected. For complete product history see page 8.

Catalogue numbers:
- **P395-025R:** SALSA MLPA Probemix P395 MEF2C-FOXG1, 25 reactions.
- **P395-050R:** SALSA MLPA Probemix P395 MEF2C-FOXG1, 50 reactions.
- **P395-100R:** SALSA MLPA Probemix P395 MEF2C-FOXG1, 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 P395 MEF2C-FOXG1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MEF2C* and *FOXG1* genes, which are associated with intellectual disability.

Intellectual disability can be provoked by different chromosomal aberrations, for example deletions of the chromosomal regions 5q14.3 and 14q12. Deletions of the 5q14.3 region mainly affect the *MEF2C* gene encoding myocyte enhancer factor 2C. It was shown that microdeletions in this gene are involved in severe intellectual disability, stereotypic movements, epilepsy, and/or cerebral malformation (Zweier 2010; Le Meur 2010; Nowakowska 2010). Another gene that has been implicated in intellectual disability is the *FOXG1* gene encoding forkhead box G1. Copy number variants (Mencarelli 2009; Kortüm 2011; OMIM #613454), as well as point mutations (Bahi-Buisson 2010; Allou 2012) have been described in literature as a potential cause of a Rett-like phenotype.

More information on *MEF2C* is available at https://www.omim.org/entry/600662
More information on *FOXG1* is available at https://www.omim.org/entry/164874

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 *MEF2C* exon numbering used in this P395-B1 MEF2C-FOXG1 product description is the exon numbering from the NG_023427.1 sequence. The *FOXG1* exon numbering used in this P395-B1 MEF2C-FOXG1 product description is the exon numbering from the NG_009367.1 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 NG 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 P395-B1 MEF2C-FOXG1 contains 42 MLPA probes with amplification products between 130 and 472 nucleotides (nt). This includes 22 probes for the *MEF2C* gene, two probes targeting sequences located upstream and two probes targeting sequences located downstream of the *MEF2C* gene, and five probes targeting the *FOXG1* gene. In addition, 11 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-fragments (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 intellectual disability. 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. 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 *FOXG1* 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

- Genetic defects in the *MEF2C* and *FOXG1* genes may also be caused by small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P395 MEF2C-FOXG1.
- 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.

Mutation databases

MEF2C: https://databases.lovd.nl/shared/genes/MEF2C
FOXG1: http://mecp2.chw.edu.au/mecp2/foxg1.php

We strongly encourage users to deposit positive results in the above databases. 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 *MEF2C* exons 9 and 11 but not exon 10) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P395-B1 MEF2C-FOXG1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*MEF2C*	*FOXG1*
64-105	Control fragments – see table in probemix content section for more information			
130 *	Reference probe 00797-L00463	5q		
137	**MEF2C probe** 16495-L18956		**Exon 11**	
142	Reference probe 07721-L07431	7p		
148 ¥ ¬	TMEM161B probe 17287-L32605		Downstream	
154	**MEF2C probe** 16496-L18957		**Exon 2**	
160	**MEF2C probe** 16497-L18958		**Exon 5**	
166 *	Reference probe 12741-L21124	21q		
172 Ø	**MEF2C probe** 16498-L18959		**Intron 8**	
178	**MEF2C probe** 16499-L18960		**Exon 5**	
185 *	Reference probe 18767-L24189	10q		
190	**MEF2C probe** 16500-L18961		**Exon 4**	
196 « ¬	**FOXG1 probe** 13756-L20771			**Upstream**
202	**MEF2C probe** 16502-L20741		**Exon 8**	
208 ¬	RASA1 probe 16501-L20775		Downstream	
216 ¥	**MEF2C probe** 16503-L32606		**Exon 4**	
228	**MEF2C probe** 16504-L26470		**Exon 9**	
235 ¥	**MEF2C probe** 16505-L32607		**Exon 3**	
242 *	Reference probe 02335-L14055	12q		
247	**MEF2C probe** 16506-L18967		**Exon 5**	
256 ¬	ADGRV1 probe 17288-L20748		Upstream	
265 Ж	**MEF2C probe** 17289-SP0470-L20749		**Exon 1**	
274 Ø	**MEF2C probe** 16507-L18968		**Intron 2**	
283 «	**FOXG1 probe** 13755-L20772			**Exon 1**
292	Reference probe 12495-L13539	1q		
304 ¥ «	**FOXG1 probe** 13757-L32608			**Exon 1**
310	**MEF2C probe** 16509-L18970		**Exon 6**	
319	Reference probe 13345-L14771	18q		
328 * ¬	**MEF2C probe** 23086-L32583		**Upstream**	
337 Ø	**MEF2C probe** 16510-L20742		**Intron 7**	
344 « ¬	**FOXG1 probe** 16850-L20773			**Upstream**
355	**MEF2C probe** 16511-L18972		**Exon 10**	
365 *	Reference probe 22419-L31604	19q		
382	**MEF2C probe** 16513-L18974		**Exon 11**	
391 ¬	CETN3 probe 16514-L18975		Upstream	
400 *	Reference probe 20685-L22083	6p		
409 Ø	**MEF2C probe** 16515-L18976		**Intron 2**	
418	**MEF2C probe** 16516-L18977		**Exon 7**	
427	Reference probe 04444-L03830	8p		
438 * Ø	**MEF2C probe** 23087-L32584		**Intron 3**	
451 * Ø	**MEF2C probe** 23089-L32586		**Intron 1**	
463 «	**FOXG1 probe** 13754-L15241			**Exon 1**
472	Reference probe 12761-L13877	4q		

[a] See section Exon numbering on page 1 for more information.

* New in version B1.
¥ Changed in version B1. Minor alteration, no change in sequence detected.
« 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.
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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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. P395-B1 probes arranged according to chromosomal location

Table 2a. *MEF2C*

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_001131005.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
256 ¬	17288-L20748	*ADGRV1* gene		CTCATAATTCCA-GTAGTTCGTGGA	217.7 kb
391 ¬	16514-L18975	*CETN3* gene		TTACCGACCTCA-GAGCTAAACTCA	1.5 **M**b
		MEF2C start codon	*430-432 (exon 3)*		
328 # ¬	23086-L32583	Upstream	1212 nt before exon 1 reverse	AGATCTTAAAAC-CTAGCTGAGTAC	0.8 kb
265 Ж #	17289-SP0470-L20749	Exon 1	450 nt and 407 nt before exon 1	AATGAGCTGCAA-43 nt spanning oligo-CCTGAAATGAAG	5.4 kb
451 Ø	23089-L32586	Intron 1	4.8 kb after exon 1 reverse	TCAATTCAGCCT-TATAGTGCATAC	11.5 kb
154	16496-L18957	Exon 2	225-224 reverse	TCTCTTCATAGA-AGTTACTTCACA	0.6 kb
274 Ø	16507-L18968	Intron 2	582 nt after exon 2 reverse	CTTCCCTACATT-AAAGCAGCGGTC	3.8 kb
409 Ø	16515-L18976	Intron 2	4.4 kb after exon 2	TACACCCAGACA-TCTTCGGGCTGC	59.2 kb
235	16505-L32607	Exon 3	321-322	GGAACTGAGCTG-TGCAAGTGCTGA	1.1 kb
438 Ø	23087-L32584	Intron 3	906 nt after exon 3 reverse	TCTCTTCCACAG-TCCAGAAACGTC	18.1 kb
190 #	16500-L18961	Exon 4	564-565	GAGATTGCGCTG-ATCATCTTCAAC	0.1 kb
216	16503-L32606	Exon 4	4 nt after exon 4	TCGTGGAGGTGA-GAGAGCATGCGT	43.0 kb
160	16497-L18958	Exon 5	447 nt before exon 5	CATGAAACTTGA-GTCCTTATACCC	0.2 kb
247	16506-L18967	Exon 5	200 nt before exon 5	GTATTTCAGACG-TTGAGAAAGAAG	0.4 kb
178	16499-L18960	Exon 5	18 nt after exon 5	ACCAAAGGTAGA-TGGCTGGTCTGC	9.1 kb
310	16509-L18970	Exon 6	981-982	TCTCCTGGTGTA-ACACATCGACCT	2.8 kb
418	16516-L18977	Exon 7	1036-1037	GTGGAGACCTCA-CGTCTGGTGCAG	16.5 kb
337 Ø	16510-L20742	Intron 7	679 nt before exon 8	TGCCTTGGTGAA-TCAGCACTTTAG	0.8 kb
202	16502-L20741	Exon 8	1195-1196	ATCTCCGAGTTC-TTATTCCACCAG	1.5 kb
172 Ø	16498-L18959	Intron 8	879 nt before exon 9	TCAGTCTGAGGA-TGTCGACCTGCT	0.9 kb
228	16504-L26470	Exon 9	1269-1270	TCGGCTCAGTCA-TTGGCTACCCCA	0.7 kb
355	16511-L18972	Exon 10	1392-1393	GCAGACCTGTCA-TCTCTGTCTGGG	5.7 kb
382	16513-L18974	Exon 11	1528-1529	ATTTATCTCAGA-GTTCAAATCTCT	0.1 kb
137	16495-L18956	Exon 11	1689-1688 reverse	CTCCCGTCGTAC-GAACTGCTACAG	526.8 kb
		MEF2C stop codon	*1819-1821 (exon 11)*		
148 ¬ #	17287-L32605	*TMEM161* gene		GGTTTCTGTAAA-CCAGGGTAATCA	864.5 kb
208 ¬	16501-L20775	*RASA1* gene		AGAAGAACGCCT-CAGGCAGGCAGG	

Table 2b. *FOXG1*

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_005249.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*494-496 (exon 1)*		
344 « ¬	16850-L20773	Upstream	559 nt before exon 1	AAATGCCAGACA-CTGGCCTGCAAG	0.2 kb
196 « ¬	13756-L20771	Upstream	349 nt before exon 1	GAGGAAGCCGGA-AATGTGAGCTAT	0.9 kb
304 «	13757-L32608	Exon 1	525-526	GAAAGAGGTGAA-AATGATCCCCAA	1.4 kb
283 «	13755-L20772	Exon 1	1907-1906 reverse	GAAATAATCAGA-CAGTCCCCCAGA	0.2 kb
463 «	13754-L15241	Exon 1	2101-2102	TCTAGGGTTGTT-TATTATTCTAAC	
		stop codon	*1961-1963 (exon 1)*		

[a] See section Exon numbering on page 1 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.

« 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.
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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
This probe's specificity 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.

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.

Complete probe sequences are available at www.mrcholland.com.

Related products

For related products, see the product page on our website.

References

- Allou L et al. (2012). 14q12 and severe Rett-like phenotypes: new clinical insights and physical mapping of *FOXG1*-regulatory elements. *Eur J Hum Genet.* 20:1216-1223.
- Bahi-Buisson N et al. (2010). Revisiting the phenotype associated with *FOXG1* mutations: two novel cases of congenital Rett variant. *Neurogenetics*. 11:241-249.
- Kortüm F et al. (2011). The core *FOXG1* syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *J Med Genet*. 48:396-406.
- Le Meur N et al. (2010). *MEF2C* haploinsufficiency caused by either microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations. *J Med Genet*. 47:22-29.
- Mencarelli MA et al. (2009). Novel *FOXG1* mutations associated with the congenital variant of Rett syndrome. *J Med Genet*. 47:49-53.
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- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.

- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.
- Zweier M et al. (2010). Mutations in *MEF2C* from the 5q14.3q15 microdeletion syndrome region are a frequent cause of severe mental retardation and diminish *MECP2* and *CDKL5* expression. *Hum Mutat*. 31:722-733.

Selected publications using SALSA MLPA Probemix P395 MEF2C-FOXG1

- Henriksen MW et al. (2013). De novo mutations in *SCN1A* are associated with classic Rett syndrome: a case report. *BMC Med Genet.* 19:184

P395 product history	
Version	*Modification*
B1	One target probe has been replaced, two target probes have been added, six reference probes have been replaced, and four target probes have been changed in length, not in sequence detected.
A2	Three probes have been changed in length, not in sequence detected.
A1	First release.

Implemented changes in the product description
Version B1-02 – 27 May 2026 (04P) - Section related products replaced with link to the website. Version B1-01 – 02 July 2021 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *FOXG1* gene updated according to new version of the NM_ reference sequence. - Changed gene name *GPR98* into *ADGRV1*. - Up - and downstream flanking probe orientation in Table 1 adjusted to *MEF2C* orientation. - Warnings added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - "Intellectual disability" has replaced the term "mental retardation" throughout this document. - Probemixes P015 MECP2 and P189 CDKL5/ARX/FOXG1 added to related probemixes.

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