Product Description
SALSA® MLPA® Probemix P336-B1 UBE3A

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 8.

Catalogue numbers
- **P336-025R:** SALSA® MLPA® Probemix P336 UBE3A, 25 reactions
- **P336-050R:** SALSA® MLPA® Probemix P336 UBE3A, 50 reactions
- **P336-100R:** SALSA® MLPA® Probemix P336 UBE3A, 100 reactions

SALSA® MLPA® Probemix P336 UBE3A (hereafter: P336 UBE3A) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P336-025R	**P336-050R**	**P336-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix 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).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, 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.

Certificate of Analysis
Information regarding 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
SALSA® MLPA® Probemix P336 UBE3A is a **research use only (RUO)** assay for the detection of deletions or duplications in the *UBE3A* gene, which is associated with Angelman syndrome (AS).

AS is a maternally inherited neurodevelopmental disorder characterized by intellectual disability, ataxia, epilepsy, minimal speech, and characteristic behavioural features. Defects in the *UBE3A* gene on chromosome 15q11.2 is one of the causes of AS. The protein encoded by this gene is ubiquitin protein ligase E3A, a part of

the ubiquitin protein degradation system. AS individuals fail to inherit a normal active maternal copy of *UBE3A*. UBE3A is subject to genomic imprinting, with predominant transcription of the maternal allele in brain.

Besides or along with mutations in the *UBE3A* gene, other known genetic causes of AS are maternal deletion of chromosome 15q11-q13, paternal chromosome 15 uniparental disomy, and 15q11 imprinting defects. Maternal duplications of 15q11-q13 lead to a distinct condition that often includes autism (Stewart et al. 2011, Hogart et al. 2009). An AS-like phenotype might be caused by aberrations in the *MTHFR* gene. Moreover, there has been evidence for linkage of 16p13 to autism (Liu et al. 2012, IMGSAC 2001).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1144/

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE

Exon numbering
The *UBE3A, MTHFR, and GABRB3* exon numbering used in this P336-B1 UBE3A product description is the exon numbering from the RefSeq transcripts NM_130839.5 (MANE), NM_005957.4 (identical to LRG_726), and NM_000814.6 (MANE). The *UBE3A* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version 04 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select NM_ sequence for this gene. 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
P336-B1 UBE3A contains 37 MLPA probes with amplification products between 129 and 454 nucleotides (nt). This includes 18 probes for the UBE3A gene, three probes for the *GABRB3* gene, and four probes for the *MTHFR* gene. In addition, twelve reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of Angelman Syndrome. 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 deviations to the indicated copy number alteration (CNA) findings might occur.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. 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 are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *UBE3A* gene are small (point) mutations, none of which will be detected by using P336 UBE3A.
- 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

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 in sequence data 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.

***UBE3A* mutation database**

https://databases.lovd.nl/shared/genes/UBE3A. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://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 *UBE3A* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. P336-B1 UBE3A

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]			
		Reference	1p36 *MTHFR*	15q11 *UBE3A*	15q12 *GABRB3*
64-105	Control fragments – see table in probemix content section for more information				
129	Reference probe 11622-L12379	10q25			
136 *	Reference probe 14277-L15947	11q14			
142	**UBE3A probe** 10883-L11553			**Exon 8**	
148	**MTHFR probe** 12083-L12971		**Exon 5**		
155 *	Reference probe 16253-L18545	19p13			
179	**UBE3A probe** 10882-L11552			**Exon 8**	
184	**GABRB3 probe** 10868-L11538				**Exon 4**
197	**UBE3A probe** 13727-L15208			**Exon 2**	
203	**GABRB3 probe** 10873-L11543				**Exon 7**
208	Reference probe 09865-L08705	13q32			
215	**UBE3A probe** 10885-L11555			**Exon 10**	
220	**MTHFR probe** 12085-L14676		**Exon 10**		
226	Reference probe 13598-L15056	9q21			
232	**UBE3A probe** 13728-L15209			**Exon 6**	
244	**UBE3A probe** 10886-L14677			**Exon 11**	
250 ¥	**GABRB3 probe** 10866-L29479				**Exon 2**
258	**UBE3A probe** 01317-L14678			**Exon 12**	
265	**MTHFR probe** 12086-L13395		**Exon 3**		
283	**UBE3A probe** 10879-L11549			**Intron 4**	
292	**UBE3A probe** 13729-L15210			**Exon 1**	
302 *	Reference probe 15881-L17974	2p16			
310	**UBE3A probe** 10884-L11554			**Exon 9**	
319	**MTHFR probe** 12087-L13396		**Exon 8**		
328	Reference probe 08543-L08544	3q24			
341	**UBE3A probe** 14083-L15682			**Exon 13**	
349	Reference probe 13442-L14897	17p13			
359 Ж	**UBE3A probe** 13731-SP0136-L15212			**Exon 7**	
365	**UBE3A probe** 13732-L16039			**Intron 3**	
372	**UBE3A probe** 14012-L15214			**Exon 5**	
381 *	Reference probe 10693-L23477	6p12			
390	**UBE3A probe** 14490-L16067			**Intron 3**	
400	Reference probe 07678-L06854	7p15			
409	**UBE3A probe** 13735-L15216			**Intron 2**	
417 ¥	**UBE3A probe** 14489-L29480			**Exon 1**	
427	Reference probe 08046-L07827	5p15			
436	**UBE3A probe** 14085-L15684			**Exon 13**	
454	Reference probe 08579-L08580	17q23			

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

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

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. Target and flanking probes arranged according to chromosomal location

Table 2a. *MTHFR*

Length (nt)	MLPA probe	*MTHFR* exon[a]	Ligation site[b] NM_005957.5	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*230-232 (Exon 2)*		
265	12086-L13395	Exon 3	15 nt before exon 3	CTCTCTTCAGAA-ACAAACCCCCTA	5.2 kb
148	12083-L12971	Exon 5	833-834	CGACATGGGCAT-CACTTGCCCCAT	1.8 kb
319	12087-L13396	Exon 8	1313-1314	CCTCTTCTACCT-GAAGAGCAAGTC	2.1 kb
220	12085-L14676	Exon 10	1634-1635	CTACTTAGAGTT-TTTCACTTCCCG	
		stop codon	*2198-2200 (Exon 12)*		

Table 2b. *GABRB3* and *UBE3A*

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		GABRB3	NM_000814.6		
		start codon	*105-107 (Exon 1)*		
250	10866-L29479	Exon 2	266-267	CGCCTAAGACCC-GACTTCGGGGGT	151.2 kb
184	10868-L11538	Exon 4	416-417	GGGATCCCTCTC-AACCTCACGCTT	53.7 kb
203	10873-L11543	Exon 7	40 nt before exon 7	TTAGTCTGCCAT-GTTGTTTCTCCA	1128.8 kb
		stop codon	*1524-1526 (Exon 9)*		
		UBE3A	NM_130839.5		
		start codon	*701-703 (Exon 3)*		
292	13729-L15210	Exon 1	100-101	GGCCTTTTCCCT-TCGCCAGGACCC	0.4 kb
417	14489-L29480	Exon 1	4 nt after exon 1	GACGACAGGTCA-GTGTTGCCGCGG	26.6 kb
197	13727-L15208	Exon 2 (Intron 1)	592-593	CAGAAGTTTGGC-GAAATATGGTAT	0.1 kb
409	13735-L15216	Intron 2 (Intron 1)	134 nt after exon 2	CTAACTACACTT-CCAAGACTGTAT	2.7 kb
365	13732-L16039	Exon 3 (Intron 1)	702-703	ATGTCACCGAAT-GGCCACAGCTTG	0.5 kb
390	14490-L16067	Intron 3 (Intron 1)	447 nt after exon 3	CAGGATGGAGAA-GCTGCACCAGTG	3.3 kb
283	10879-L11549	Exon 4 (Intron 2)	131 nt after exon 4	ATGGGAGATAGG-AACATACCTACT	29.8 kb
372	14012-L15214	Exon 5 (3)	968-969	AGAAAGGAGCAA-GCTCAGCTTACC	4.6 kb
232 #	13728-L15209	Exon 6 (4)	1873-1874	AATCACAATGAA-GAAGATGATGAA	10.6 kb
359 Ж	13731-SP0136-L15212	Exon 7 (5)	2380-2381; 2407-2408	GAAGGAGAACAA-27 nt spanning oligo-GAATTTTTTCAG	3.6 kb
179	10882-L11552	Exon 8 (6)	2523-2524	AACTGAGGGTCA-GTTTACTCTGAT	0.1 kb
142 #	10883-L11553	Exon 8 (6)	2612-2613	TCTACAGGAAGC-TAATGGGGAAAA	0.8 kb
310 #	10884-L11554	Exon 9 (7)	2735-2736	TCACTTTCCAGA-TATCACAGACAG	1.4 kb
215 #	10885-L11555	Exon 10 (8)	2912-2913	TTCATATGGTGA-CCAATGAATCTC	0.2 kb
244	10886-L14677	Exon 11 (9)	3052-3053	TCTGTTCTGATT-AGGTGAGGTACT	14.2 kb
258	01317-L14678	Exon 12 (10)	3084-3085	TCATTCATTTAC-AGATGAACAGAA	1.8 kb
341	14083-L15682	Exon 13 (11)	4064-4065	GTCTTGCAATGA-ACTGTTTCAGTA	0.4 kb
436	14085-L15684	Exon 13 (11)	4433-4434	TACTTAATCATA-CAGTAAGCTGAC	
		stop codon	*3317-3319 (Exon 13)*		

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

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.

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related products

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

References

- Hogart A et al. (2009). Chromosome 15q11–13 duplication syndrome brain reveals epigenetic alterations in gene expression not predicted from copy number. *J Med Genet*, 46(2), 86-93.
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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.
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- 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.

Selected publications using P336 UBE3A

- Aguilera C et al. (2017). Novel intragenic deletions within the UBE3A gene in two unrelated patients with Angelman syndrome: case report and review of the literature. *BMC med genet*, 18(1), 137.
- Aguilera C et al. (2019). Identification of a de novo splicing variant in the Coffin–Siris gene, SMARCE 1, in a patient with Angelman-like syndrome. *Mol genet genom med*, 7(1), e00511.
- Beleza-Meireles A et al. (2011). Novel deletion encompassing exons 5-12 of the UBE3A gene in a girl with Angelman syndrome. *Eur J Med Genet*. 54(3):348-50.
- Beygo J et al. (2019). Update of the EMQN/ACGS best practice guidelines for molecular analysis of Prader-Willi and Angelman syndromes. *Eur J Hum Genet*, 27(9), 1326-1340.
- Cali F et al. (2010). Novel deletion of the E3A ubiquitin protein ligase gene detected by multiplex ligation-dependent probe amplification in a patient with Angelman syndrome. *Exp Mol Med*. 42(12):842-8.
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P336 product history	
Version	*Modification*
B1	Four probes detecting 16p13 and the *MTHFR* mutation-specific probe have been removed, four reference probes have been replaced, and two probes have been adjusted in length.
A2	One reference probe has been removed and QDX2 fragments have been added.
A1	First release.

Implemented changes in the product description
Version B1-04 — 08 December 2025 (05P) - Product description adapted to a new template. - Exon numbering, NM_reference sequence and ligation sites of the probes targeting the *UBE3A* gene have been updated to the MANE in section: Exon numbering and Table 1 & 2, no change in actual target sites. - NM_reference sequence and ligation sites of the probes targeting the *GABRB3* gene have been updated to the MANE in section: Exon numbering and Table 2, no change in actual target sites. - Related SALSA MLPA products section replaced with reference to product page on website. - MANE link added to section: Gene structure and transcript variants. Version B1-03 — 27 March 2025 (02P) - Exon numbering, NM_reference sequence and ligation sites of the probes targeting the *UBE3A* gene have been changed. Version B1-02 — 11 December 2024 (02P) - The term 'mental retardation' is considered outdated and was removed as synonym of 'intellectual disability'. Version B1-01 — 29 May 2020 (02P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *MTHFR* and *GABRB3* genes updated according to new version of the NM_ reference sequences. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200