Product Description
SALSA® MLPA® Probemix P262-B2 GHI

To be used with the MLPA General Protocol.

Version B2
As compared to version B1, two probe lengths have been adjusted. For complete product history see page 8.

Catalogue numbers:
- **P262-025R:** SALSA MLPA Probemix P262 GHI, 25 reactions.
- **P262-050R:** SALSA MLPA Probemix P262 GHI, 50 reactions.
- **P262-100R:** SALSA MLPA Probemix P262 GHI, 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 P262 GHI is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GHR*, *JAK2*, *IGF1*, and *STAT5B* genes, which are associated with growth hormone insensitivity (GHI).

GHI is characterized by severe short stature, normal to elevated serum levels of growth hormone (GH) and resistance to exogenous GH therapy. The aetiology of GHI is classically associated with mutations in the GH receptor (*GHR*) gene or with mutations affecting the signalling cascade of the *GHR* (OMIM 245590). Intracellular signalling molecules activated by GH belong to the Janus kinase-signal transducer and activator of transcription 5B (*JAK2-STAT5B*) pathway. Amongst others, this pathway activates the insulin-like growth factor (*IGF1*), which is implicated in the regulation of protein turnover and exerts potent mitogenic and differentiating effects on most cell types.

There are two genomic isoforms of the *GHR* gene in humans: a full-length isoform (GHRfl) and an isoform lacking exon 3 (GHRd3). The distribution of these genotypes differs among populations, with the frequency for GHRfl/GHRfl ranging from 35–53%, for GHRfl/GHRd3 between 33–58%, and for GHRd3/GHRd3 ranging from 7-26%. Growth hormone is used to increase height in short children who are not deficient in growth hormone receptor, but its efficacy varies widely between individuals. Dos Santos et al. (2004, Nat Genet.) found that the GHRd3 isoform was associated with 1.7 to 2 times more growth acceleration induced by growth hormone than the full-length isoform.

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 *GHR*, *JAK2*, *IGF1*, and *STAT5B* exon numbering used in this P262-B2 GHI product description is the exon numbering from the NG_011688.2, LRG_612, NG_011713.1, and LRG_192 sequences, respectively. 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 LRG 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 P262-B2 GHI contains 50 MLPA probes with amplification products between 130 and 494 nucleotides (nt). This includes 11 probes for the *GHR* gene, one probe for each exon and two probes for exon 10, 15 probes for the *JAK2* gene for 15 different exons, four probes for the *IGF1* gene, and 11 for the *STAT5B* gene. In addition, nine 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 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 growth hormone insensitivity. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Selection of reference samples for SALSA MLPA Probemix P262-B2 GHI:

The choice of reference samples is important for the correct determination of copy numbers using MLPA. The P262-B2 GHI probemix contains one probe that detects exon 3 of the *GHR* gene (at 344 nt), which, apart from the 'normal' two copies, can be either heterozygous and homozygous deleted in a substantial portion of the population. For data analysis, reference samples should be chosen that contain two copies of the sequence detected by the exon 3 probe.

MRC-Holland is unfortunately not able to supply suitable reference samples. In order to select suitable reference samples for your experiments, we recommend testing DNA from 16 healthy individuals in the first experiment. These should preferably be samples that are derived from the same type of tissue and purified by the same method as your samples to be tested. Analysis of these 16 samples using the average of these 16 samples will allow one to identify the samples that have two copies of *GHR* exon 3. The correct choice can be confirmed by re-analysis of the data using only these samples as reference sample.

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. 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

- In most populations, the major cause of genetic defects in the *GHR*, *JAK2*, *IGF1*, and *STAT5B* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P262 GHI.
- 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.

OLVG mutation database

https://databases.lovd.nl/shared/genes/. 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 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 *GHR* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P262-B2 GHI

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*GHR*	*JAK2*	*IGF1*	*STAT5B*
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 09978-L10437	19p				
145	Reference probe 19948-L29905	10q				
149	**JAK2 probe** 07443-L29395			**Exon 5**		
157	**IGF1 probe** 07438-L29906				**Exon 5**	
163	**JAK2 probe** 07452-L30067			**Exon 23**		
172	**GHR probe** 07429-L29397		**Exon 7**			
178	**JAK2 probe** 07441-L29398			**Exon 3**		
184	**STAT5B probe** 07457-L29435					**Exon 4**
192	**JAK2 probe** 07445-L29399			**Exon 7**		
199	**JAK2 probe** 21155-L29440			**Exon 25**		
204	Reference probe 18317-L27218	15q				
209 «	**GHR probe** 07422-L29400		**Exon 1**			
215	**JAK2 probe** 07439-L29649			**Exon 1**		
222	**GHR probe** 07426-L29403		**Exon 4**			
228	**JAK2 probe** 07444-L29402			**Exon 6**		
234	**GHR probe** 07432-L29438		**Exon 10**			
240 «	**STAT5B probe** 07454-L29404					**Exon 1**
247	**STAT5B probe** 07460-L09388					**Exon 12**
254	Reference probe 05223-L26271	2q				
264 «	**JAK2 probe** 07449-L09389			**Exon 16**		
270	**STAT5B probe** 07459-L30118					**Exon 7**
278	**STAT5B probe** 21156-L30119					**Exon 18**
285	**GHR probe** 07431-L30120		**Exon 9**			
292	**IGF1 probe** 07435-L29406				**Exon 2**	
301	**IGF1 probe** 07434-L09967				**Exon 1**	
310	**STAT5B probe** 07465-L07113					**Exon 19**
321	**JAK2 probe** 21152-L29436			**Exon 2**		
331	Reference probe 18779-L29907	3p				
337	**STAT5B probe** 07462-L29407					**Exon 15**
344 +	**GHR probe** 20708-L30093		**Exon 3**			
352	**JAK2 probe** 07446-L29409			**Exon 9**		
361	**GHR probe** 21150-L29432		**Exon 2**			
369	Reference probe 18067-L26511	16q				
376 «	**JAK2 probe** 07450-L29410			**Exon 19**		
382	**GHR probe** 07428-L29411		**Exon 6**			
391 «	**JAK2 probe** 07451-L29412			**Exon 21**		
399	**STAT5B probe** 07458-L29413					**Exon 6**
408	**IGF1 probe** 21159-L29908				**Exon 3**	
414	**STAT5B probe** 07455-L29909					**Exon 2**
421	**JAK2 probe** 07442-L29910			**Exon 4**		
427	**GHR probe** 07427-L30117		**Exon 5**			
431 ¥	**STAT5B probe** 07461-L29912					**Exon 13**
439	Reference probe 10093-L29913	8q				
445	**STAT5B probe** 21153-L30154					**Exon 3**
454	**GHR probe** 07430-L07078		**Exon 8**			
462	**JAK2 probe** 07447-L29414			**Exon 12**		
472	**JAK2 probe** 21157-L29441			**Exon 14**		
478 ¥	Reference probe 09205-L29656	18p				
486	**GHR probe** 07433-L29415		**Exon 10**			
494	Reference probe 19137-L26747	21q				

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

¥ Changed in version B2. 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.

+ This probe is specific for *GHR* exon 3. It will only give a signal when the GHRfl isoform is present on one or both alleles. Please read the section 'Selection of reference samples' on page 3.

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. P262-B2 probes arranged according to chromosomal location

Table 2a. *GHR*

Length (nt)	SALSA MLPA probe	*GHR* exon[a]	Ligation site NM_000163.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*529-531 (Exon 2)*		
209 «	07422-L29400	Exon 1	508-509	GATCAGAGGCGA-AGCTCGGAGGTA	141.9 kb
361	21150-L29432	Exon 2	548-549	CTGGCAGCTGCT-GTTGACCTTGGC	64.2 kb
344 +	20708-L30093	Exon 3	996 nt after exon 3	AACTTTGTCCTT-TACAACATGATA	58.9 kb
222	07426-L29403	Exon 4	757-758	ATCATGGTACAA-AGAACCTAGGAC	6.0 kb
427	07427-L30117	Exon 5	901-900, reverse	CTTGATACAATA-AGGTATCCAGAT	4.9 kb
382	07428-L29411	Exon 6	1048-1049	ATATCCAAGTGA-GATGGGAAGCAC	11.3 kb
172	07429-L29397	Exon 7	1189-1190	TGTACTCATTGA-AAGTGGATAAGG	2.3 kb
454	07430-L07078	Exon 8	1403-1402, reverse	TCCACACCTACC-TTTGCTGTTTAG	4.6 kb
285	07431-L30120	Exon 9	1430-1431	TCTGCCCCCAGT-TCCAGTTCCAAA	0.8 kb
234	07432-L29438	Exon 10	1877-1878	CAGTGTTATCCA-AGCAGAGAAAAA	0.1 kb
486	07433-L29415	Exon 10	1988-1989	CATCGACTTTTA-TGCCCAGGTGAG	
		stop codon	*2443-2445 on 10)*		

Table 2b. *JAK2*

Length (nt)	SALSA MLPA probe	*JAK2* exon[a]	Ligation site NM_004972.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*495-497 (Exon 3)*		
215	07439-L29649	Exon 1	215-216	CTGGTCCTCGCT-GCCGAGGGATGT	0.5 kb
321	21152-L29436	Exon 2	412-411, reverse	CAGTTCACATCT-TGTTCCTGTTGC	35.7 kb
178	07441-L29398	Exon 3	268 nt before exon 3	GTGGAGTGCGGA-GGTTTGCTGCAG	8.2 kb
421	07442-L29910	Exon 4	816-815, reverse	ATTATGCCTGGT-TGACTCATCTAT	14.6 kb
149	07443-L29395	Exon 5	876-877	ATTGCAGTGGCA-GCAACAGAGCCT	6.3 kb
228	07444-L29402	Exon 6	1036-1035, reverse	CTAACACTGCCA-TCCCAAGACATT	4.0 kb
192	07445-L29399	Exon 7	1300-1301	CTACACAGAGAA-ATTTGAAGTAAA	10.2 kb
352	07446-L29409	Exon 9	1647-1648	ATTACCTCTGTA-AAGAAGTAGCAC	5.0 kb
462	07447-L29414	Exon 12	2044-2045	AACGAATGGTGT-TTCTGATGTACC	3.8 kb
472	21157-L29441	Exon 14	2291-2292	GAAGCAGCAAGT-ATGATGAGCAAG	4.6 kb
264 «	07449-L09389	Exon 16	2567-2566, reverse	ATGAAAGGAGGA-TTTCCTGTCTTC	3.5 kb
376«	07450-L29410	Exon 19	3013-3014	TGAAGACCGGGA-TCCTACACAGTT	8.8 kb
391«	07451-L29412	Exon 21	3378-3379	CTCAGATATGCA-AGGTAACTAATA	32.5 kb
163	07452-L30067	Exon 23	3575-3576	GAATCACTGACA-GAGAGCAAGTTT	4.2 kb
199	21155-L29440	Exon 25	4296-4297	AATACCTTGGCA-TCTTGTGTGATG	
		stop codon	*3891-3893 on 25)*		

Table 2c. *IGF1*

Length (nt)	SALSA MLPA probe	*IGF1* exon[a]	Ligation site NM_001111283.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*183-185 (Exon 1)*		
301	07434-L09967	Exon 1	190-191	AGCAATGGGAAA-AATCAGCAGTCT	4.6 kb
292	07435-L29406	Exon 2	274-275	GTCCTCCTCGCA-TCTCTTCTACCT	56.2 kb
408	21159-L29908	Exon 3	502-503	GAGGCTGGAGAT-GTATTGCGCACC	23.4 kb
157	07438-L29906	Exon 5	7044-7045	ATTTCCCCTGCT-ACTTTGAAACCA	
		stop codon	*657-659 (Exon 5)*		

Table 2d. *STAT5B*

Length (nt)	SALSA MLPA probe	*STAT5B* exon[a]	Ligation site NM_012448.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*155-157 (Exon 2)*		
240 «	07454-L29404	Exon 1	358 nt after exon 1	CGCCTCACACCT-TCACGTTGCAAT	43.8 kb
414	07455-L29909	Exon 2	211-212	CATCAGATGCAA-GCGTTATATGGC	4.4 kb
445	21153-L30154	Exon 3	312-313	TAATCCACAGGA-GAACATTAAGGC	2.8 kb
184 #	07457-L29435	Exon 4	490-491	ATCCGCCATATA-TTGTACAATGAA	4.9 kb
399	07458-L29413	Exon 6	95 nt before exon 6	TATCTGAGCCCA-GGAGGGTCTCGC	0.8 kb
270 #	07459-L30118	Exon 7	199 nt after exon 7	TGCTGTCCTGGA-GATGGGACAGGG	3.1 kb
247	07460-L09388	Exon 12	1580-1581	GCAGCCAGGACA-ACAATGCGACGG	3.9 kb
431 #	07461-L29912	Exon 13	1669-1670	GTGCTGTGGCCA-CAGCTGTGTGAG	1.8 kb
337	07462-L29407	Exon 15	49 nt before exon 15	GGTCTTCTCTCT-GGCATCGTAAGT	8.0 kb
278 #	21156-L30119	Exon 18	2370-2371	TCCCCAGGCTCA-CTATAACATGTA	2.3 kb
310	07465-L07113	Exon 19	4212-4213	GAGGAGCAGGCT-ACCCGCATCCCA	
		stop codon	*2516-2518 (Exon 19)*		

[a] See section Exon numbering on page 2 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.

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.

« 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.

+ This probe is specific for GHR exon 3. It will only give a signal when the GHRfl isoform is present on one or both alleles. Please read the section Data Analysis on page 2 concerning selection of reference samples!

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 SALSA MLPA probemixes

P018 SHOX	Contains probes for *SHOX* (Léri-Weill Dyschondrosteosis).
P026 Sotos	Contains probes for *NSD1* and *NFIX* (Sotos syndrome).
P216 GHD mix 1	Contains probes for genes involved in growth hormone deficiency.
P217 IGF1R	Contains probes for *IGF1R, IGFBP3,* and *IGFALS*.

References

- Dos Santos C et al. (2004). A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nat Genet*, 36(7), 720-724.
- 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.

Selected publications using SALSA MLPA Probemix P262 GHI

- Gorbenko del Blanco D et al. (2012). Growth hormone insensitivity syndrome caused by a heterozygous GHR mutation: phenotypic variability due to moderation by nonsense-mediated decay. *Clin Endocrinol*. 76:706-12.
- Jabari M (2019). A novel homozygous point mutation and deletion in exon 3 of growth hormone receptor causes laron syndrome: A case study. *Imam J Appl Sci*, 4(2), 83.
- Mul D et al. (2012). A mosaic de novo duplication of 17q21-25 is associated with growth hormone insensitivity, disturbed in vitro CD28 mediated signalling and decreased STAT5B, PI3K and NF-kB activation. *Eur J Endocrinol*. 166:743-52.

P262 product history	
Version	*Modification*
B2	Two probe lengths have been adjusted.
B1	All reference probes have been replaced and five have been added, one *STAT5B* probe has been removed and several probe lengths have been adjusted
A2	The 88 and 96 nt control fragments have been replaced, two control fragments at 100 and 105 nt have been included (QDX2)
A1	First release.

Implemented changes in the product description

Version B2-01 – 23 June 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 GHR, STAT5B and IGF1 genes updated according to new version of the NM_ reference sequence.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 11 – 01 September 2017 (55)
- Warning added in Table 1, 209 nt probe 07422-L29400, 240 nt probe 07454-L29404, 264 nt probe 07449-L09389, 376 nt probe 07450-L29410, 391 nt probe 07451-L29412, and 472 nt probe 21157-L29441.
- Probe numbers and lengths for the exon 18 and exon 19 probes of STAT5B corrected in Table 2d.
- Various minor textual changes.

Version 10 – 10 August 2017 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes on pages 1 and 2.

Version 09 – 11 December 2015 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Exon numbering of the IGF1 gene has been changed in Table 1 and Table 2c.
- Manufacturer's address adjusted.

Version 08 – 24 March 2015 (54)
- Electropherogram pictures using the old MLPA buffer removed.
- Warning on GHR exon numbering in table 2 removed.

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