Product Description
SALSA® MLPA® Probemix P257-A3 TERT-DKC1

To be used with the MLPA General Protocol.

Version A3
For complete product history see page 7.

Catalogue numbers:
- **P257-025R:** SALSA MLPA Probemix P257 TERT-DKC1, 25 reactions.
- **P257-050R:** SALSA MLPA Probemix P257 TERT-DKC1, 50 reactions.
- **P257-100R:** SALSA MLPA Probemix P257 TERT-DKC1, 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 P257 TERT-DKC1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *DKC1*, *TERT* and *TERC* genes, which are associated with dyskeratosis congenita.

Dyskeratosis congenita (DC) is characterised by multiple features including mucocutaneous abnormalities, bone marrow failure and an increased predisposition to cancer (Dokal 2011). While DC exhibits marked clinical and genetic heterogeneity, the wide range of disease manifestations caused by this syndrome have a defective telomere maintenance in common. DC is caused by mutations is several genes, three of which are covered by the P257 TERT-DKC1 probemix, with the mode of inheritance varying by gene. X-linked recessive DC (~25% of DC) is characterized by mutations in the *DKC1* gene (Dokal et al. 2015), which encodes dyskerin, a component of H/ACA small nucleolar ribonucleoprotein (snoRNP) particles (Dokal 2011). Mutations in *TERC*, the RNA component, and *TERT*, the enzymatic component of telomerase, each constitute ~5% of all DC cases (Dokal et al. 2015). Whereas *TERC* mutations lead to autosomal dominant DC, *TERT* mutations can lead to either autosomal dominant or autosomal recessive DC. DC has a prevalence of 1 in a million, and 90% of all mutations leading to this syndrome come from single nucleotide variants, although deletions (4.8%) and duplications (2%) have also been observed (AlSabbagh 2020).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK22301/.

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 *DKC1* exon numbering used in this P257-A3 TERT-DKC1 product description is the exon numbering from the LRG_55 sequence.

The *TERT* exon numbering used in this P257-A3 TERT-DKC1 product description is the exon numbering from the LRG_343 sequence.

The *TERC* exon numbering used in this P257-A3 TERT-DKC1 product description is the exon numbering from the LRG_347 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 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 P257-A3 TERT-DKC1 contains 47 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes at least one probe for each of the 15 exons of the *DKC1* gene. This probemix also contains at least one probe for each exon of the *TERT* gene, with the exception of exon 1. Finally, two probes for the single *TERC* exon and two probes flanking the *TERC* gene are present. In addition, eleven 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 of the same sex 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 dyskeratosis congenita. It is recommended to use samples of the same sex to facilitate interpretation. 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 when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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 *DKC1*, *TERT* and *TERC* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P257 TERT-DKC1.
- 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.

Telomerase mutation database

http://telomerase.asu.edu/. We strongly encourage users to deposit positive results in the Telomerase Database. 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 *DKC1* exons 10 and 12 but not exon 11) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P257-A3 TERT-DKC1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*DKC1*	*TERT*	*TERC*
64-105	Control fragments – see table in probemix content section for more information				
130 *	Reference probe 00797-L00463	5q			
136 ¥	**TERC probe** 08640-L30932				**Exon 1**
142 ¥	**DKC1 probe** 19511-L30933		**Exon 2**		
148	**DKC1 probe** 08626-L08642		**Exon 6**		
155	Reference probe 04566-L03955	16q			
160	**DKC1 probe** 08630-L08646		**Exon 10**		
166	**TERT probe** 08649-L08665			**Exon 9**	
172	Reference probe 02797-L06029	3p			
178	**DKC1 probe** 08635-L08651		**Exon 15**		
184	**TERT probe** 08647-L09621			**Exon 7**	
190	**TERT probe** 08655-L08671			**Exon 15**	
196	**DKC1 probe** 08624-L08640		**Exon 4**		
202	Reference probe 02654-L02121	11q			
208 ¬	**TERC probe** 08637-L08653				**0.4 kb upstream**
214	**DKC1 probe** 08627-L08643		**Exon 7**		
220	**TERT probe** 08644-L08660			**Exon 4**	
228	**TERT probe** 08652-L08668			**Exon 12**	
232	Reference probe 08612-L08624	8p			
244	**DKC1 probe** 08628-L08644		**Exon 8**		
250	**TERT probe** 08643-L09620			**Exon 2**	
258 ¥	**TERT probe** 08657-L30935			**Exon 16**	
265	Reference probe 00474-L00388	20p			
274	**TERT probe** 08650-L08666			**Exon 10**	
284	**DKC1 probe** 08636-L08652		**Exon 15**		
293	**TERT probe** 08654-L08670			**Exon 14**	
300	**TERC probe** 08639-L09617				**Exon 1**
310	Reference probe 01293-L00838	9p			
319 ¥	**DKC1 probe** 18761-L24340		**Exon 9**		
328	**TERT probe** 08651-L08667			**Exon 11**	
337	**DKC1 probe** 08632-L08648		**Exon 12**		
346	**DKC1 probe** 08621-L08637		**Exon 1**		
355 *	Reference probe 21479-L25060	13q			
364	**TERT probe** 08653-L08669			**Exon 13**	
373	**DKC1 probe** 08631-L08647		**Exon 11**		
382 ¬	**TERC probe** 08638-L08654				**0.3 kb downstream**
391	**TERT probe** 08656-L08672			**Exon 16**	
400	Reference probe 04538-L03927	2q			
409	**DKC1 probe** 08634-L08650		**Exon 14**		
418	**DKC1 probe** 08623-L08639		**Exon 3**		
426	**TERT probe** 08648-L08664			**Exon 8**	
437	**TERT probe** 03761-L02477			**Exon 3**	
445	**TERT probe** 08645-L08661			**Exon 5**	
454	Reference probe 08579-L08580	17q			
463	**TERT probe** 08646-L08662			**Exon 6**	
472	**DKC1 probe** 08625-L08641		**Exon 5**		
481	**DKC1 probe** 08633-L08649		**Exon 13**		
490 *	Reference probe 12461-L21828	22q			

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

* New in version A3.

¥ Changed in version A3. Minor alteration, no change in sequence detected.

¬ 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.

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. P257-A3 probes arranged according to chromosomal location

Table 2a. *DKC1* gene

Length (nt)	SALSA MLPA probe	*DKC1* exon[a]	Ligation site NM_001363.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*103-105 (Exon 1)*		
346	08621-L08637	Exon 1	2 nt after exon 1	GATGCGGAAGGT-AAGGGCTGCAGG	2.0 kb
142	19511-L30933	Exon 2	180-181	CCAGAAGAAGAT-GTAGCCGTGAGT	0.5 kb
418	08623-L08639	Exon 3	18 nt before exon 3	TTAATAGGTTAT-ATTTCTGTCCTG	0.6 kb
196	08624-L08640	Exon 4	2 nt after exon 4	ACTATATCAGGT-AAGTGTTGGGAG	0.4 kb
472	08625-L08641	Exon 5	498-499	ACTGGTTGTTTA-ATCGTGTGCATA	0.7 kb
148	08626-L08642	Exon 6	585-586	CGGCTGCACAAT-GCTATTGAAGGG	0.3 kb
214	08627-L08643	Exon 7	700-701	TGAGGACCATCT-ACGAGAGCAAAA	1.0 kb
244	08628-L08644	Exon 8	809-810	CCTTGGTTTGTT-ATTGGGAGTTGG	0.8 kb
319	18761-L24340	Exon 9	903-904	CATGATGTGCTT-GATGCTCAGTGG	1.6 kb
160	08630-L08646	Exon 10	1092-1093	ATTGAGGTCAAT-CAGGAGATTGTG	2.4 kb
373	08631-L08647	Exon 11	1190-1191	CCATGGTATAGT-AGCCAAGATCAA	1.5 kb
337	08632-L08648	Exon 12	1301-1302	CCTTCTGGACAA-GCATGGGAAGCC	0.5 kb
481	08633-L08649	Exon 13	17 nt before exon 13	AATTTGACCTAT-TGCTACCTCTTT	1.0 kb
409	08634-L08650	Exon 14	1464-1465	AGTGAGAGTGAA-AGTGACGAGACT	0.5 kb
178	08635-L08651	Exon 15	34 nt before exon 15	TTGTTAGTGGAT-GGTATCTGTGAG	0.2 kb
284	08636-L08652	Exon 15	1777-1778	ATTTTAGCTGCT-ACTTTGAGACCT	
		stop codon	*1645-1647 (Exon 15)*		

Table 2b. *TERT* gene

Length (nt)	SALSA MLPA probe	*TERT* exon[a]	Ligation site NM_198253.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*80-82 (Exon 1)*		
	No probe	Exon 1			
250	08643-L09620	Exon 2	1547-1548	GCTTCCTCAGGA-ACACCAAGAAGT	10.9 kb
437	03761-L02477	Exon 3	1765-1766	TCTTTCTTTTAT-GTCACGGAGACC	2.3 kb
220	08644-L08660	Exon 4	2012-2013	GAGCCAGAACGT-TCCGCAGAGAAA	0.8 kb
445	08645-L08661	Exon 5	2134-2135	GGCCTGGACGAT-ATCCACAGGGCC	0.6 kb
463	08646-L08662	Exon 6	2277-2278	CGCCAGCATCAT-CAAACCCCAGAA	6.5 kb
184	08647-L09621	Exon 7	2405-2406	ACATGCGACAGT-TCGTGGCTCACC	1.1 kb
426	08648-L08664	Exon 8	2517-2518	CCTACGCTTCAT-GTGCCACCACGC	2.6 kb
166	08649-L08665	Exon 9	2639-2640	AGAACAAGCTGT-TTGCGGGGATTC	2.0 kb
274	08650-L08666	Exon 10	2690-2691	TGGATGATTTCT-TGTTGGTGACAC	2.0 kb
328	08651-L08667	Exon 11	2809-2810	TTCCCTGTAGAA-GACGAGGCCCTG	4.0 kb
228	08652-L08668	Exon 12	3000-3001	GCGTCGCAAACT-CTTTGGGGTCTT	1.9 kb
364	08653-L08669	Exon 13	3083-3084	GCACCAACATCT-ACAAGATCCTCC	3.3 kb
293	08654-L08670	Exon 14	3159-3160	GCAAGTTTGGAA-GAACCCCACATT	1.0 kb
190	08655-L08671	Exon 15	3344-3345	ACCGTGTCACCT-ACGTGCCACTCC	0.6 kb
391	08656-L08672	Exon 16	13 nt before exon 16	ACGGAGTCTGAT-TTTGGCCCCGCA	0.1 kb
258	08657-L30935	Exon 16	3468-3469	CTTCAAGACCAT-CCTGGACTGATG	
		stop codon	*3476-3478 (Exon 16)*		

Table 2c. *TERC* gene

Length (nt)	SALSA MLPA probe	*TERC* exon[a]	Ligation site NR_001566.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
208 ¬	08637-L08653	Upstream	421 nt before exon 1	GAGCCGAGACAA-GATTCTGCTGTA	0.5 kb
300	08639-L09617	Exon 1	41-42	GGTGGCCATTTT-TTGTCTAACCCT	0.3 kb
136	08640-L30932	Exon 1	314-315	GAGTTGGGCTCT-GTCAGCCGCGGG	0.4 kb
382 ¬	08638-L08654	Downstream	275 nt after exon 1	AGATTTTGTTGA-GGTTTTTGCTTC	

[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.

¬ 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.

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.

References

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- 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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P257 product history	
Version	*Modification*
A3	Three reference probes have been replaced and several probe lengths have been adjusted. In addition, one Y chromosome specific control probe has been removed.
A2	The 88 and 96 nt control fragments have been replaced and two control fragments at 100 and 105 nt have been included (QDX2).
A1	First release.

Implemented changes in the product description

Version A3-02 – 01 February 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *DKC1* and *TERT* genes updated according to new version of the NM_ reference sequence.

Version A3-01 – 30 April 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Warning removed in Table 1 and 2 about location of probes within a strong CpG island.

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