Ligase-65

Ligase-65 is used in MLPA, but can also be used for other purposes. The following Ligase-65 reagent kits are available.

Lig-5a
1x 550 µl SALSA Ligase-65
1x 880 µl SALSA MLPA buffer[1]
5x 360 µl Ligase buffer A
2 x 880 µl Ligase buffer B

Lig-10
2 x 550 µl SALSA Ligase-65

Lig-50
10 x 550 µl SALSA Ligase-65

Ligase-65 is a NAD-dependent ligase enzyme purified from the bacterial strain MRCH-0065. It is used in the MLPA reaction as described in *Nucleic Acid Research (2002) 30, e57*, however Ligase-65 may also be useful for other purposes. In MLPA, a mixture of MLPA buffer, Ligase buffer A and Ligase buffer B is used for incubation with Ligase-65. A protocol for MLPA reactions is available on www.mlpa.com. The Ligase-65 enzyme can be used up to 65 °C in MLPA. In contrast to other thermophilic ligases, ligase-65 is more easily denatured at high temperatures. In the MLPA reaction, a 5 minutes incubation at 98 °C is used for inactivation of the enzyme, but virtually all activity is destroyed during a 5 minutes 95 °C treatment.

Ligase-65 can ligate 90% of the oligonucleotides that are annealed to adjacent sites of a DNA target sequence, within 1.5 minute at 54 °C – 65 °C in a volume of 40 µl buffer, when 1 µl of this Ligase-65 preparation is used. We recommend a tenfold longer incubation time in order to reach ≥ 99 % completion of the ligation reaction. Incubation for longer times, or with larger amounts of Ligase than needed to reach 99% completion, will result in a decreased difference in ligation between perfectly matched and imperfectly matched probe-target complexes. Using the above mentioned conditions, the ligation of perfectly matched probe-target hybrids should reach 99%≥ completion. The ligation of a hybrid in which the 3' nucleotide of one of the probe oligonucleotides has a mismatch with the target sequence, should be less than 2% complete. Avoid T/G mismatches. DNA oligonucleotides hybridised to a RNA target will not be ligated by Ligase-65.

None Hazardous ingredients

None of the ingredients are hazardous in the amount present in a reagent kit as defined in the Hazard Communication Standard (HCS). None of the ingredients are of human or animal origin. None of the ingredients are derived from pathogenic bacteria. A statement 'MSDS not required' for SALSA MLPA probemixes and reagent kits is available on our website www.mlpa.com.

Stability

Ligase Buffer A is a 10 x concentrated NAD solution. Distribute ligase-A buffer in smaller aliquots to avoid more than 15 freeze-thaw cycles. Repeated freeze-thaw cycles will destroy the NAD.

Storage and shelf life

All components of the SALSA® MLPA® reagent kit must be stored directly upon arrival at -15 °C to -25 °C in the dark and in the original package. When stored under the recommended conditions, a shelf life of at least 1 year is guaranteed. See the labels on each vial for the expiry date of each individual reagent.

SALSA® MLPA® reagents are sold by MRC-Holland for research purposes. This kit is not CE/FDA certified for use in diagnostic procedures. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

More information

Website : www.mlpa.com
E-mail : info@mlpa.com (information & technical questions); order@mlpa.com (for orders)
Mail : MRC-Holland bv; Willem Schoutenstraat 6, 1057 DN Amsterdam, the Netherlands

[1] Since January 2013 a new MLPA buffer is supplied to make MLPA more robust, which can be recognised by its yellow label.