QUICK-STICK LIGASE

Shipping: On Dry/Blue Ice

Batch No.: See vial

Catalog numbers

BIO-27027 : 50 reactions

BIO-27028 : 100 reactions

BIOLINE

A Meridian Life Science® Company

Store at –20°C

Storage and stability:
Quick-Stick Ligase is shipped on Dry/Blue Ice and should be stored at -20°C upon receipt. Repeated freeze/thaw cycles should be avoided.

Expiry
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Please refer to the material safety data sheet for further information.

Quality control specifications:
Quick-Stick Ligase activity is assayed by testing for a single band of the products of both *Hind*III and *Eco*RV-cut Lambda DNA. The ligated DNA is then re-cut to ensure no alteration of restriction pattern. Quick-Stick Ligase tested for exo- and endonuclease and RNase activities contamination prior to release.

Notes:
Research use only.

Description

Bioline Quick-Stick Ligase is designed for the ligation of both blunt and sticky-ended fragments in just 15 and 5 minutes respectively at room temperature (25°C). The Quick-Stick Ligase kit can be used to perform linker ligation, re-ligation of linearized plasmids and ligation of double-stranded oligonucleotides into vectors. The kit enables rapid ligation of up to 100ng of fragments from most sources including PCR fragments, plasmids, cosmids, genomic, phage and viral DNA into prokaryotic or eukaryotic plasmid vectors and bacteriophage lambda vectors.

Components

Product Name	50 reactions	100 reactions
Quick-Stick Ligase	1x50µl	1x100µl
4xQS Buffer	1x250µl	2x250µl
DNA Dilution Buffer	1x1.25ml	1x1.25ml

Quick-Stick Ligation Protocol:

1) Assemble the reaction in a microcentrifuge tube at room temperature in the order outlined below:

   a) Combine the vector and the insert in the appropriate ratio to make up no more than 100ng of DNA.
   b) Adjust volume to 14µl with ddH_2O.
   c) Add 1µl of QS Ligase.
   d) Add 5µl of 4x QS Buffer (always vortex before use).

2) Mix thoroughly by pipetting.

3) **Incubate at room temperature for 5 minutes for cohesive ends, or for 15 minutes for blunt ends.**

4) *Optional:* run 2.5-5µl of ligation mixture on to an agarose gel to check ligation efficiency before subjecting the DNA to transformation reactions.

5) Product is ready for transformation (or storage at -20°C).
   No heat inactivation required.

Notes

- Always vortex 4 x QS Buffer before use.
- Avoid multiple freeze/thawing
- As Mg/ATP ratio is crucial for successful reaction, avoid the use of DNA solutions with concentration of EDTA higher than 0.1mM. Use the **DNA Dilution Buffer** provided for dissolving DNA pellets or dilution of concentrated DNA solutions for following ligation.

General Considerations:

1. **Preparation of Vector and Insert DNA molecules:** For ligation to occur efficiently the ends of the DNA molecules must be compatible. Prepare the Vector and insert DNA molecules obtained by restriction digest, PCR amplification, or other physical/enzymatic methods. In order to separate the vector and insert from other contaminating molecules, the DNA to be ligated should be purified, by using electrophoretic, physical or organic extraction followed by ethanol precipitation. After purification, the DNA should be quantified.

We recommend using the Bioline SureClean reagent (BIO-37042), a novel, inexpensive solution, for column-free nucleic-acid purification.

2. **Dephosphorylation of Vector DNA molecules:** When blunt-ended ligations are carried out, it may be beneficial to dephosphorylate the vector (remove the 5′-phosphate groups) to prevent self-ligation.
3. **Cloning of PCR Amplification Products:** A simple way of preparing the fragments for ligation is by incorporating the restriction enzyme sites near the 5′-end of the PCR primers, and digesting following amplification. Alternatively, for use with a dephosphorylated vector, phosphate groups can be added to either the 5′-end of primers used in the PCR, or to the amplification products themselves.
4. **Ratio of Vector DNA:Insert DNA:** The ideal vector-insert, or phage-insert ratio may be determined empirically, but is generally in the molar range of 1:3 for vector:insert ligations, or 8:1 for vector arm:insert phage ligations. For the first cloning of PCR-product, the ratio recommended for vector-insert ligation is 1/10 to 1/100.
5. **ATP Concentration:** ATP is present in the 4x Quick-Stick Reaction Buffer and is optimised to favour best performance of Quick-Stick Ligase.
6. **Enzymatic Treatment of Ligation Reactions:** Following ligation, DNA molecules may be treated with any enzyme without danger of interference from the 4x Quick-Stick Reaction Buffer.
7. **Time of Ligation:** 5 minutes at room temperature (25°C) is sufficient time to ligate either 99% of *Hind*III-digested lambda DNA (sticky-ended fragments), or 80% of *Eco*RV-digested lambda DNA (blunt-ended fragments), from 100ng of starting material. 15 minutes at room temperature is the recommended time for complete blunt-end ligation. The reaction time can be extended for up to overnight at 16°C incubation temperature.

Website: www.bioline.com/ email: info@bioline.com

Calculation of Molarity of Ends :

Molarity = [(mg/ml) + (base pairs X 650 daltons)] X 2 ends

Example:

- To calculate the molarity of ends for a linearised 4Kb vector that has a concentration of 200ng/µl:
  Molarity = [(0.20µg/µl) / (4000 X 650 daltons)] X 2 ends = 154nM

- To calculate the molarity of ends if you put 50ng of this vector in a 20ml ligation reaction:

  50ng in 20µl = 0.0025µg/µl

  Molarity = [(0.0025µg/µl) / (4000 X 650 daltons)] X 2 ends = 1.92nM

- Determine the amount of a 1Kb insert to be added to achieve a 3:1 insert:vector ratio:

  3 X 1.92nM = 5.76nM
  [(? µg/µl) / (1000 X 650 daltons)] X 2 ends = 5.76nM
  0.0019µg/µl X 20µl = 0.038µg ≈ 40ng
  Please note: Answer at stage two of this calculation is divided by 10^9 to convert from Daltons (M) to nM.

- Is this below the DNA limit of 50µg/ml ?
  (50ng Vector + 40ng Insert) / 20µl = 4.5µg/ml

A guide to some commonly used plasmids:

- 1µg of 1000 bp DNA = 3.04pmol ends,
- 1µg of linear pUC18/19 DNA = 1.14pmol ends,
- 1µg of linear pBR322 DNA = 0.7pmol ends,
- 1µg of linear SV40 DNA = 0.58pmol ends,
- 1µg of linear PhiX 174 DNA = 0.56pmol ends,
- 1µg of linear M13mp18/19 DNA = 0.42pmol ends,
- 1µg of l phage DNA = 0.06pmol ends.

Troubleshooting

Problem	Possible Cause	Recommendation
Few/No Recombinant Colonies recovered	Incorrect ratio of vector to insert.	Verify the concentration of the vector and the insert. Perform the ligation using a different ratio of vector to insert.
	Experimental DNA contains an inhibitor of ligation.	Ensure DNA is free of contaminants (e.g. excess salts, EDTA, proteins, phenol, etc.) that may inhibit the ligation reaction. Gel purify and/or extract the vector and insert prior to ligation.
	Vector not fully digested.	Analyze digest by electrophoresis, repeat digest for a longer time period or add more restriction enzyme.
	Incompatible ends	Check that the ends of the DNA molecules are compatible.
	Cloned sequence is not tolerated in the host.	If possible, check the target sequence for strong promoters or other potentially toxic elements, as well as inverted repeats. In case the product of a cloned gene is detrimental to a host, use promoters with a very low expression background.
	Not enough DNA transformed.	Verify the amount of DNA added to the competent cells.
	Inactive competent cells	Verify that the competent cells are active. Use the undigested vector as a positive control.
	Ligation into a dephosphorylated vector	Check that the oligonucleotides used in PCR amplification have 5′ phosphate groups, or incorporate the appropriate restriction sites.
High number of Non-recombinant Colonies	Vector not dephosphorylated	Remove 5′-phosphate groups from vector molecules
	Vector not linearized	Check linearization of vector molecules
	Antibiotic Inactive too low concentration	Supply medium with fresh antibiotic

Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

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