

# V. Cloning and Ligation

**Product Overview\***

**Ligation**

**Cloning Kits**

**Competent Cells**

\* for Cloning Service see I. Services

## Product Overview

### Ligation

Prod.No.	Product	Pack-size	Price in €
M3431.0030	Alligator Ligation Kit with competent cells	30 reactions	370.67
M3431.0060	Alligator Ligation Kit with competent cells	60 reactions	708.82
M3027.1010	T4 DNA-Ligase	10000 units	39.02
M3027.2000	T4 DNA-Ligase	2000 units	11.71

### Cloning Kits

Prod.No.	Product	Pack-size	Price in €
M3164.0020	pMBL-T/A Cloning Kit	20 reactions	186.64
M3164.0040	pMBL-T/A Cloning Kit	40 reactions	336.85
M3457.0010	QuickClone Kit	10 reactions	198.35

### Competent Cells

Prod.No.	Product	Pack-size	Price in €
M3434.0010	Chemical competent cells (>10E8)	10 transformations	115.75
M3434.0020	Chemical competent cells (>10E8)	20 transformations	206.79
C4293.0500	Solubiliser for protein expression (solution)	500 mL	81.94
C4293.2500	Solubiliser for protein expression (solution)	5 x 500 mL	240.61
C4294.0001	Solubiliser for protein expression (powder)	1 L	46.18
C4294.0005	Solubiliser for protein expression (powder)	5 x 1 L	150.22

### Competent Cells

Your cloning and transformation results could only be as good as your competent cells - that is nothing new.  
Preparing high quality competent cells is not only very time consuming but also not always easy - also nothing new.

**But what is new? Our selection of high quality competent *E. coli* cells!**

**Chemical competent cells for routine transformations: *E. coli* TZ101<sup>□</sup>**

- Strain suitable for most applications.
- Blue/white screening possible.
- Due to *recA* mutation reduction of recombination events.
- *endA* mutation ensures improved DNA plasmid quality.

**Transformation efficiency > 1 x 10<sup>8</sup> cfu/μg**

**Genotype:**

F<sup>+</sup>/ *endA1 hsdR17 glnV44 thi-1 recA1 gyrA relA1* <sup>□</sup>(*lacIZYA-argF*)U169 *deoR* (p80*dlac*<sup>□</sup>(*lacZ*)M15)

Prod.No.	Product	Pack-size	Price in €
M3434.0010	Chemical competent cells (>10E8)	10 transformations	115.75
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## V. Cloning and Ligation

### Ligation

#### T4 DNA Ligase

Prod.No.	Product	Pack-size	Price in €
M3027.2000	T4 DNA-Ligase	2000 units	11.71
M3027.1010	T4 DNA-Ligase	10000 units	39.02

T4 DNA Ligase catalyses the formation of a phosphodiester bonds between 5' phosphate and 3' hydroxyl termini in duplex DNA/RNA. This enzyme can join blunt end and cohesive end termini, repair single stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids. Cohesive end ligation: for most cohesive end ligations, a 30 minute incubation at 20°C is sufficient. Incubations at 16°C for 4-16 hours are routinely used for the majority of applications. Ligation of blunt ends and single-base pair overhang fragments requires more enzyme to achieve the same extent of ligation as cohesive end DNA fragments. Ligation may be enhanced by addition of PEG, or by reducing the rATP concentration. ATP is an essential cofactor for the reaction.

#### Concentration

100 CEL units/μL  
(one Weiss unit equals 67 "cohesive end ligation units").

#### Reaction buffer

50 mM Tris-HCl pH 7.8, 10mM MgCl<sub>2</sub>, 10mM DTT, 1 mM ATP

#### Unit definition

One unit is defined as the amount of enzyme required to give 50% ligation of *Hind* III fragments of lambda DNA in 30 minutes at 16°C at 5' termini concentration of 0.12 μM (300μg/mL). One Cohesive End Ligation Unit equals 0.015 Weiss units. One Weiss unit equals 67 Cohesive End Ligation Units.

#### Comments

Purified free of endonucleases.

#### Assay conditions

Ligation of cohesive ends: 0.01 unit T4 DNA Ligase will ligate a minimum of 90% of 1μg of lambda *Pst* I fragments in a 20μL reaction medium overnight at 16°C. Ligation of blunt ends: 0.3 units of T4 DNA Ligase will ligate a minimum of 70% of 1μg *Hae* III pUC8 fragments in a 20μL reaction medium after 20 h at 16°C.

#### Storage and shipping

Store at -20°C, shipped refrigerated

#### Alligator™ Very Fast Ligationskit + competent cells (Optimised mixture of T4 Ligase, buffer and ATP shipped with competent cells)

Prod.No.	Product	Pack-size	Price in €
M3431.0030	Alligator Ligation Kit with competent cells	30 reactions	370.67
M3431.0060	Alligator Ligation Kit with competent cells	60 reactions	708.82

The Genaxxon bioscience Alligator™ DNA Ligation kit is a simple one component system that allows to perform DNA ligation reactions very rapidly. The kit uses T4 DNA ligase and an optimised buffer system. Due to the extremely high efficiency of the ligation reaction, conventional overnight incubations are no longer necessary.

Various kinds of ligations with blunt or sticky end DNA fragments as well as T/A overhangs can be performed in a minimum time - in most cases, one minute reaction time leads to sufficient efficiency. The reaction can proceed directly to bacterial transformation.

By using the Genaxxon bioscience competent cells shipped with Alligator™ (Cat#: M3431) the whole ligation and transformation procedure can be done within 20 minutes.

#### Comment

This kit is a very fast ligation kit, that enables ligation in 1 minute at RT. After ligation the following steps are done as usual. For your convenience this kit is shipped with competent cells, that are an improvement of existing *E.coli* HB5.

#### Storage and shipping

This kit is shipped on dry-ice. Please store competent cells at -80°C. The ligase containing component should be kept at -20°C.

## Cloning Kits

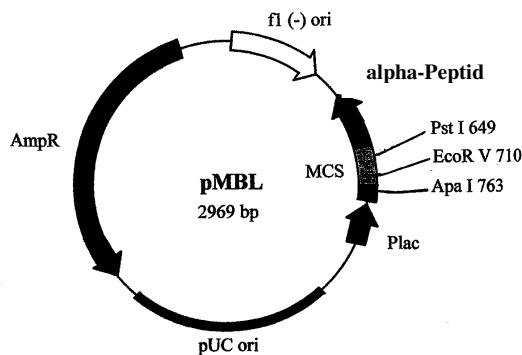
### pMBL T/A Cloning Kit

(T/A Cloning Kit for cloning of DNA-fragments with A-overhang)

Prod.No.	Product	Pack-size	Price in €
M3164.0020	pMBL-T/A Cloning Kit	20 reactions	186.64
M3164.0040	pMBL-T/A Cloning Kit	40 reactions	336.85

The pMBL-T/A Cloning Kit is a convenient system for the cloning of PCR products. The pMBL-T/A Vector is prepared by cutting the pMBL vector with *EcoR V* and adding a 3' terminal thymidine to both ends. These single 3'-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product into the plasmid by preventing recircularization of the vector and providing a compatible overhang for ligation of PCR products. The high copy number pMBL vector contains a T3 RNA polymerase promoter in the 3'-region of the MCS for in vitro RNA transcription. Insertional inactivation of the alpha-peptide allows recombinant clones to be directly identified by color screening on indicator plates. The pMBL-T/A Cloning Kit includes T4 DNA Ligase and buffer for ligation in up to 1 to 2 hours.

#### Comment



#### Storage and shipping

Kit is shipped on blue ice. Please store at -20°C.

Protocol and further details can be found on the Genaxxon bioscience webpage:  
[www.genaxxon.com](http://www.genaxxon.com)

### QuickClone Kit

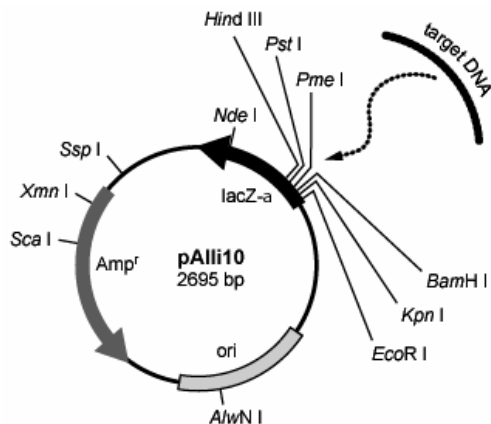
(Blunt end cloning kit for fast and reliable cloning of DNA from different sources)

Prod.No.	Product	Pack-size	Price in €
M3457.0010	QuickClone Kit	10 reactions	198.35

The Genaxxon bioscience QuickClone Kit was developed to allow cloning of DNA fragments from various origins (sticky ends, PCR-products with or without T/A overhangs, etc.) in a two-step reaction that takes less than 60 min including transformation.

The kit comprises two buffers: buffer 1 renders all DNA termini blunt and phosphorylated. Buffer 2 mediates the insertion of the fragment (now blunt ended) into a suitable vector, completely prepared and included in buffer 2. The kit is supplied with optimised competent cells *E. coli* TZ101<sup>+</sup> allowing high-efficiency transformation of the ligated DNA fragments.

#### Comment



#### Storage and shipping

Kit is shipped on dry ice. Please store at -80°C.

Protocol and further details can be found on the Genaxxon bioscience webpage:  
[www.genaxxon.com](http://www.genaxxon.com)