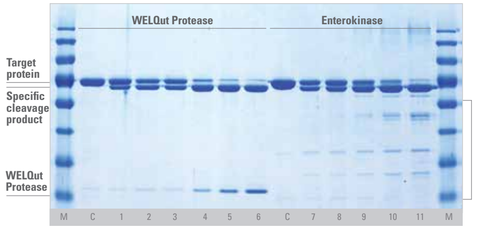
Benefit from superior features of novel Thermo Scientific WELQut protease

Highly specific proteolysis of N-terminal His-tag with WELQut Protease

Target protein (Klenow Fragment, exo⁻, containing WELQut or Enterokinase recognition sequence) was treated with WELQut Protease and Enterokinase according to the recommendations provided by the suppliers.

Various enzyme/substrate amount ratios were tested and reaction products were analyzed by SDS-PAGE.

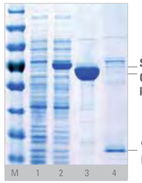


M – Thermo Scientific PageRuler Prestained Protein Ladder (#26616)
C – Control-uncut target protein.
1- 6 – various WELQut/target protein amount ratios, u/µg
1- 1:100, **2** - 1:50, **3** - 1:40, **4** - 1:20, **5** - 1:10, **6** - 1:5
7- 11 – various Enterokinase/target protein amount ratios, µg/µg
7- 1:100, **8** - 1:40, **9** - 1:20, **10** - 1:10, **11** - 1:5

Unspecific cleavage products

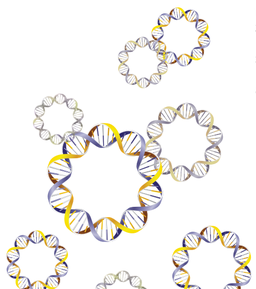
Ideal for on-column proteolysis reactions

Due to built-in His tag WELQut is suitable for on-column digestion streamlining your protein expression experiments. The target protein (Klenow Fragment, exo⁻ cloned into pLATE52) was purified and digested using IMAC in a column setup (Thermo Scientific HisPurTM Ni-NTA Spin Columns).



Substrate
Cleavage product
WELQut protease

M – PageRuler™ Prestained Protein Ladder (#26616)
1 – Negative control (*E. coli* ER2566/pLATE52-Cat before induction)
2 – Lysate load (*E. coli* ER2566/pLATE52-Klenow Fragment, exo⁻ after 3 hour induction with 0.1 mM IPTG
3 – Eluate I (after enzymatic His tag removal, Klenow Fragment, exo⁻ is eluted from the IMAC sorbent)
4 – Eluate II, imidazole eluted proteins (efficiencies of cleavage reaction and removal of WELQut from reaction mixture)

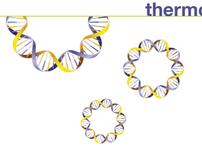


Order details

Product	Order #	Preps
aLICator LIC Cloning and Expression Kit 1	K1241	20 rxns
aLICator LIC Cloning and Expression Kit 2	K1251	20 rxns
aLICator LIC Cloning and Expression Kit 3	K1261	20 rxns
aLICator LIC Cloning and Expression Kit 4	K1281	20 rxns
aLICator LIC Cloning and Expression Set 1	K1271	30 rxns
aLICator LIC Cloning and Expression Set 2*	K1291	30 rxns
WELQut Protease	EO0861	500 u

*sample with the WELQut Protease included is available

• Learn more at
thermoscientific.com/onebio



Europe
Customer Service
cs.molbio.eu@thermofisher.com
Technical Support
ts.molbio.eu@thermofisher.com
Tel 00800 222 00 888
Fax 00800 222 00 889

United States
Customer Service
cs.molbio@thermofisher.com
Technical Support
ts.molbio@thermofisher.com
Tel 800 235 9880 (press 1)
Fax 800 292 6088

Canada
Customer Service
cs.molbio@thermofisher.com
Technical Support
ts.molbio@thermofisher.com
Tel 800 340 9026 (press 1)
Fax 800 472 8322

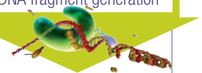
US Literature # MB022912K01U
Euro Literature # MB_2012_10

Everything for your Thermo Scientific **cloning and protein expression** workflow

Sample preparation

GeneJET Purification Kits
Plasmid DNA • Genomic DNA • Total RNA

DNA fragment generation



Fast Digest Restriction Enzyme
Phusion DNA polymerase • dNTPs

Electrophoresis

DNA Ladders • RNA Ladders • Loading Buffers • Running Buffers • Agarose

Modification of DNA ends

FastAP Alkaline Thermosensitive Phosphabates • Fast DNA End Repair Kit • T4 DNA Polymerase • Klenow Fragment

Cloning

CloneJet PCR Cloning Kit • Rapid DNA Ligation Kit • T4 DNA Ligase • aLICator LIC Cloning and Expression System

thermoscientific.com/onebio

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

Successful cloning & robust protein expression

• High protein yields • Tight control of protein expression – ideal for toxic genes • His-tag allows for easy detection and purification of expressed proteins • Removal of the N-terminal His-tag with enterokinase or WELQut Protease

Thermo SCIENTIFIC

Streamlined cloning for successful protein expression

Thermo Scientific aLICator LIC Cloning and Expression System is designed for fast and efficient cloning with subsequent tightly regulated gene expression in *E. coli.* pLATE bacterial expression vectors are designed for the high levels of target protein expression in concert with minimized basal (uninduced) expression.

Applications

- Directional PCR product cloning
- Tightly regulated protein expression
- Expression of toxic genes
- Highly specific removal of N-terminal His tags from recombinant protein using novel WELQut Protease.

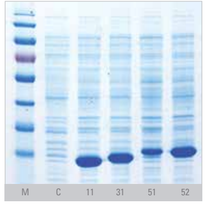
The aLICator system consists of six kits based on the pLATE series of bacterial expression vectors. For proteins with a known preference for either the N- or C-terminal 6xHis-tag position, using the appropriate N- or C-terminal kit is recommended. When the protein structure and features are not well known, it is recommended to use a set - clone into all three vectors and determine the most compatible vector for further research. Following protein affinity purification, amino-terminal tags can be removed either via Enterokinase (EK), DDDDK^ or a novel WELQut (WQ), WELQ^ cleavage sites located immediately N-terminal to the target protein.

aLICator LIC Cloning Kits		Features
aLICator LIC Cloning and Expression Kits	Kit 1 Untaged	For untagged protein expression, pLATE11 vector
	Kit 2 N-terminal His-tag/EK	For N-terminal His-tag protein expression, pLATE51 vector, Enterokinase cleavage
	Kit 3 C-terminal His-tag	For C-terminal His-tag protein expression, pLATE31 vector
	Kit 4 N-terminal His-tag/WQ	For N-terminal His-tag protein expression, pLATE52 vector, WELQut Protease cleavage
aLICator LIC Cloning and Expression Sets	Set 1 All-in-One/EK	For identification of optimal expression vector. Choice of untagged, C- or N-terminal His-tag protein expression in pLATE11, pLATE31 and pLATE51 (with Enterokinase site for tag removal) vectors respectively
	Set 2 All-in-One/WQ	For identification of optimal expression vector. Choice of untagged, C- or N-terminal His-tag protein expression in pLATE11, pLATE31 and pLATE52 (with WELQut Protease site for tag removal) vectors respectively

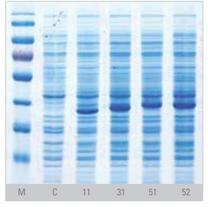
Expression of different proteins within pLATE vectors

Fail-safe cloning and robust expression of different proteins varying in size, origin, toxicity.

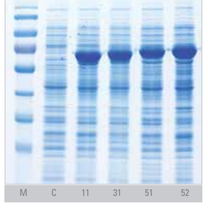
26 kDa chloramphenicol acetyltransferase



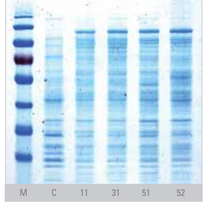
37 kDa restriction endonuclease



75 kDa Klenow Fragment, exo-



115 kDa ß-galactosidase

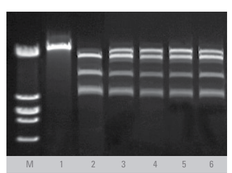


The aLICator system allows for expression of variety of target proteins including difficult to deal with. SDS-PAGE images demonstrate the successful expression of large, 115 kDa protein as well as restriction enzyme or Klenow Fragment, exo - which are toxic to the living cells.

C – Unninduced E.coli ER2566 cells (pLATE11 + insert) Indduced ER2566 cells with recombinant plasmid:
11 – pLATE11 vector
31 – pLATE31 vector
51 – pLATE51 vector
52 – pLATE52 vector
M - Thermo Scientific PageRuler Prestained Protein Ladder (#26616)

Expression of toxic genes

Tightly regulated expression in pLATE vectors allows for cloning of toxic genes in E.coli cells.



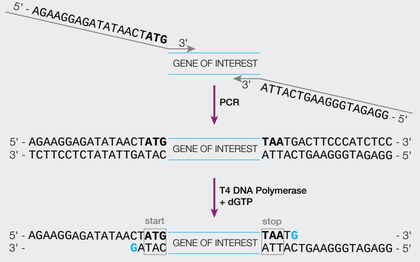
Tightly regulated gene expression with pLATE vectors allows for synthesis of toxic genes in *E. coli.* The toxic Cfr9I R gene was cloned into pLATE vectors and expressed in *E. coli* DH10B cells. Expression was induced with IPTG for three hours. Bacterial cells were collected, normalized according to optical density and sonicated. Cell-free extract of each clone was assessed for Cfr9I activity through digestion of Lambda DNA (lanes 3-6)

M – Thermo Scientific GeneRuler High Range DNA Ladder (#SM1353)
1 – Lambda DNA
2 – Lambda DNA / Cfr91
3,4,5,6 – cell free extract with Cfr91 expressed within pLATE11, pLATE31, pLATE51 and pLATE52 vectors respectively.

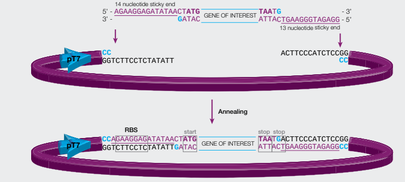
LIC Cloning principle

The aLICator cloning and expression system uses robust directional LIC cloning technology to streamline and facilitate the process of insert cloning into the expression vector.

- >95% clones with desired insert
- Directional cloning
- 15 minutes procedure
- Restriction digestion and ligation is not involved



The LIC method uses 3'→5' exonuclease activity of the bacteriophage T4 DNA polymerase to create specific 14 – 21 base single-stranded overhangs on the LIC vectors and DNA inserts (2). Exonuclease activity of T4 DNA polymerase removes nucleotides from the 3' ends of the DNA and the polymerase activity restores the chain. When reaction contains only one of four dNTPs, the dGTP, equilibrium of 3'→5'-exonuclease and 5'→3'-polymerase activity is achieved at the site of the first occurrence of dCTP.



After annealing, the pLATE vector and insert are transformed into competent *E. coli* cells without ligation reaction. Covalent bond formation at the vector-insert junctions occurs within the cell to yield circular plasmid.

pLATE vector elements

pLATE expression vectors use elements from bacteriophage T7 to control expression of heterologous genes in *E. coli.* The expression of the gene of interest is driven by a strong bacteriophage T7 promoter that is specifically recognized by T7 RNA polymerase. To express the gene of interest, *E. coli* strains such as BL21 (DE3), HMS 174 (DE3) must be used, in which expression of T7 RNA polymerase gene is under the control of an inducible promoter such as lacUV5. After IPTG induction, theT7 RNA polymerase is expressed within the cell, and starts transcription of genes under the T7 promoter.