Data sheet

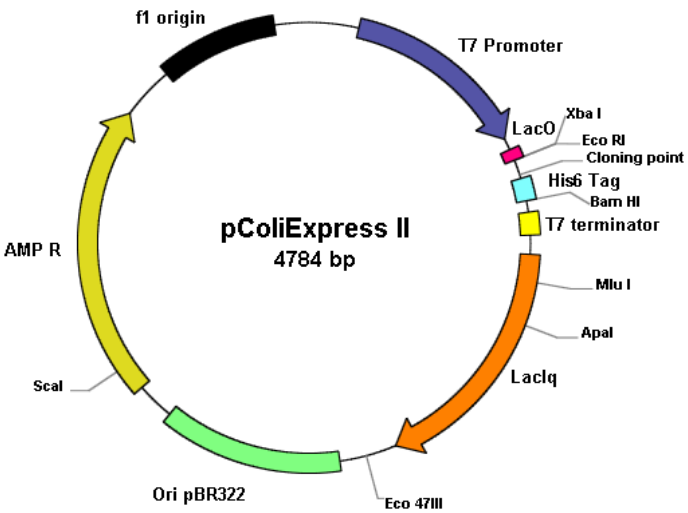
pColiExpress™ II Ligation Cloning & Expression Kit

Cat. No: BE006 (20 reactions)
Cat. No: BE007 (20 reactions)-Plus

Description

pColiExpress II is a DNA cloning vector for protein expression in *E.coli*. All family pColiExpress DNA cloning vectors are based in a technology which allows efficiently cloning DNA fragments and the quick production of a large quantity of any desired protein.

pColiExpress II contains all elements required for expression of **large quantity of any desired protein** by T7 RNA Polymerase Inducible System and a His_6 tag at COOH end that allow the protein purification. The vector has also an f1 origin of replication, an ampicillin resistance cassette, and a pBR322 origin of replication.



Unique restriction sites are showed.

Kit Components

Components	BE006	BE007[1]
pColiExpress II (50 ng/µL)[2]	20 µL	20 µL
5X T4 DNA Ligase Buffer	200 µL	200 µL
T4 DNA Ligase (5 U/µL)	20 µL	20 µL
Control Insert DNA (30 ng/µL)	10 µL	10 µL
pColiExpress II Control[3] (50 ng/µL)	5 µL	5 µL
***Eco*R V (10 U/µL)**	-	**20 µL**
***Hind* III (10 U/µL)**	-	**20 µL**
10X Buffer C	-	**1.5 mL**

(1) You need add at 5´ end Hind III cleavage site in forward primer and Eco RV cleavage site at 5´ end in reverse primer.
(2) Linearized DNA Vector.
(3) Circular vector. Empty DNA Vector.

pColiExpress vector family is a **ready to use** vector for a highly efficient cloning procedure. The vector is linearized, just for ligate with your PCR amplified with the recommending primers. Experimental background is less than 2%.

Features	
T7 Promoter	4605-4621
Lac O	4627-4645
T7 transcription start	4621
His Tag	4710-4727
T7 terminator	4749-4796
LacIq	96-1175
Ori pBR322	2970-2356
Ampicillin resistance gene (ORF)	3133-3990
F1 Origin	4562-4115

(Continued on reverse side)

Assay procedure

PCR restriction enzyme digestion

1. Your PCR reaction must be cleaned or loaded into agarose gel before.
2. Spin restriction enzymes and Buffer C to collect content at the bottom and set up the reaction as described below.

Match Reaction	Cloning Reaction
PCR cleaned	1 µg
10x Buffer C	7 µL
Eco RV (10 U/µL)	0.7 µL
Hind III (10 U/µL)	0.7 µL
Water (*Molecular Biology grade*)	up 70 µL

Cloning

3. Your PCR digestion must be cleaned and quantified before. Clean Easy PCR Purification kit (AN0063) is recommended.
4. Spin all kit components to collect content at the bottom of the tubes before set up the reaction.
5. Set up reaction as described below.

Match Reaction	Cloning Reaction	Control Reaction	Background Reaction
pColiExpress II vector (50 ng/µL)	1 µL	1µL	1 µL
5x T4 DNA Ligase Buffer	2 µL	2 µL	2 µL
PCR Product*	X µL	-	-
Control Insert DNA	-	1 µL	-
T4 DNA ligase (5 Weiss units/µL)	1 µL	1µL	1 µL
Water (*Molecular Biology grade*)	up 10 µL	up 10 µL	up 10 µL

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**Relation vector: insert 1:5 is recommended*.

6. Mix the reactions by pipetting.
7. Incubate one hour at Room Temperature (20-25ºC).

Transformation

8. Centrifuge the tubes containing the reactions to collect content at the bottom of the tube. Add 15 µL of each reaction to a sterile 1.5 mL microcentrifuge tube on ice. Set up another tube on ice with 50 pg uncut plasmid for determination of the transformation efficiency of the competent cells (not supplied).
9. Place the competent cells in an ice bath until just thawed (about 10 to 15 minutes). Mix the cells by gently flicking the tube with your fingertips.
10. Carefully transfer 50 µL of cells into each tube prepared in Step 8.
11. Gently flick the tubes to mix and place them on ice for 30 minutes.
12. Heat-shock the cells for exactly 45 seconds in a water bath at exactly 42°C.
13. Immediately return the tubes to ice for 2 minutes and plate all transformation mix onto pre-warmed LB ampicillin plates. Incubate the plates overnight (12–16 hours) at 37°C.