艾科瑞生物
Accurate Biology

Instruction for Use

OK Clon DNA Ligation Kit II

AG11807 AG11808
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This Product is designated for accurate, fast, efficient and directional cloning of one or more fragments of DNA into any vector within 10 minutes. The OK Clon Enzyme has 3'-5' Exonuclease activity, which cleaves nucleotides from the 3' ends of linearized DNA fragments to form 15- 20 BP length complementary sticky ends, which can be ligated accurately and efficiently by annealing. This 15-20 BP length overlap could be engineered by designing primers for amplification of the desired sequences.

2. Kit Information

Kit Name	Cat. No	Specification
OK Clon DNA Ligation Kit II	AG11807	10 rxns / 10 μl
OK Clon DNA Ligation Kit II	AG11808	50 rxns / 10 μl

3. Transportation and Storage

Storage	Store at –20°C
Transportation	Transport at –20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	AG11807	AG11808
2.5X OK Clon Master Mix	40 μl	100 μl x 2pc
Linearized Control vector (50 ng/μl)	5 μl	5 μl
2 kb Positive Control Insert (80 ng/μl)	5 μl	5 μl

5. Protocol

5.1 Experimental Preparation

Design PCR primers per the target gene of interest with 15-20 BP extensions (5') that are complementary to the ends of the linearized vector. Amplify the target gene of interest via high fidelity PCR and purify the PCR product. Linearize the vector by restriction enzyme digestion or inverse PCR and purify the product.

Prepare Competent Cells for following cloning experiment.

5.2 Reagent Preparation

The final reaction volume in this protocol is 10 μl. The volumes given here may be scaled for larger or smaller reaction volume.

Components	Final Concentration	Volume
2.5X OK Clon Master Mix	1 X	4 μl
Linearized Vector	50 ng ~ 200 ng	-
Insert DNA fragment	5 ng ~ 200 ng	-
RNase Free Water	-	Up to 10 μl

*1: Prepare all components beside the 2.5X OK Clon Master Mix into one premix tube, then add 2.5X OK Clon Master Mix lastly. Gently pipetting 3-5 times, Mix thoroughly.
(Avoid inserting the pipette tip too deep into the solution)
*2: Input of 50-100 ng for fragments<10kb; Input of 50-200 ng for fragments>10kb.
*3: Input of 10-50 ng for inserts<0.5kb; Input of 50-100 ng for inserts between 0.5 kb - 10kb ; Input of 50-200 ng for inserts>10kb.
Recommended insert to vector molar ratio as below.

	Insert Length < Vector Length	Insert Length ≥ Vector Length
Single Fragment	2:1	1:1
	(Adjust within the range of 2:1 ~ 5:1, for Insert Length<0.5kb)	
Multi Fragment	Recommended molar ratio of each insert to vector is 2:1	

Note: All ratios listed are molar ratios.
Reference for Calculation of molar ratio based on the mass and length of insert and vector is as below.

Insert amount (ng) = A x B/C x Vector amount (ng)
While A= Molar ratio of insert to vector
B= Insert Length (BP)
C= Vector Length (BP)
EX: Insert 1000 BP fragment into 4000 BP vector, Vector amount 100 ng
Then insert amount = 2 x 1000 BP/4000BP x 100 ng = 50 ng

*4: Recommended volume of control vector and insert volume is 1 μl of each.
*5: For larger volume of vector and insert (>6 μl of vector + insert), Double the amount of 2.5X OK Clon Master Mix and then add RNase Free Water for up to 20 μl.

5.3 Reaction Program

The parameters below are offered as a guideline and may be modified as necessary for optimal results.

Temperature	Time
50°C	10 min*
4°C	-

*: 10 min reaction time is recommended for most of experiments.
If the recombination efficiency is not satisfactory, adjust the reaction time within 8-10 min.
For complex 15-20 BP of sequence homology (High GC ratio), prolong the reaction time to 12 min; For simplified 15-20 BP of sequence homology (High AT ratio), reduce the reaction time to 8 min.

6. Transformation

[Take the transformation method of chemically competent cells as an example]

Thaw Competent Cells in an ice bath just before use (**NOTE:** Competent cells cannot be stored for a long time after thawing, and cannot be frozen and thawed repeatedly);

Pipette 5 μl of the recombination products to 100 μl of competent cells, flip the tube for several times to mix thoroughly (**DO NOT VOTEX!**), and then keep in the ice bath for 30 min (**NOTE:** The volume of transformation products should not be more than 1/10 of the volume of competent cells);

Incubate cells for 45 sec at 42°C; Return to the ice bath for 2 min immediately;

Add 900 μl of SOC medium to the tube. Shake at 37°C for 1h at 200 rpm;

Centrifuge the culture at 6,000 rpm for 5 min, discard 900 μl of supernatant. Then, re-suspend the pellet with 100 μl of remaining medium and plate it on an agar plate with appropriate selection of antibiotic.

Incubate at 37°C for 12-16 hours.environment.

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