



Hieff Clone™ Universal One Step Cloning Kit

Product description

Hieff Clone[™] Universal One Step Cloning Kit is a simple, fast, and highly efficient cloning technology and enables directional insertion of any amplified DNA product into any linearized vector at any site. Firstly, the vector is linearized at the cloning site. A small sequence overlapped with each end of the cloning site is added onto the insert through PCR. The insert and the linearized vector, with overlapped sequences of 15 bp - 20 bp on both 5'- and 3'-end, respectively, are mixed in an appropriate ratio and incubated with recombinase at 50°C for 5-15 min.

Hieff Clone™ Universal one step cloning kit is a novel cloning Kit, independent of DNA ligase, significantly reducing the vself-ligated colonies and bringing a true positive rate > 95%.

Components

Components No.	Name	10922ES20 (20T)	10922ES50 (50T)
10922-A	Hieff Clone™ Universal Enzyme Premix	200 μL	500 μL
10922-B	500 bp Control Insert (25 ng/μL)	5 μL	5 μL
10922-C	pUC 19 Control Vector, linearized (50 ng/μL)	5 μL	5 μL

Specifications

Cloning Process	Seamless cloning
Control	Positive control
Segments	Up to 6 fragments
Product type	Seamless Cloning and Assembly Kit

Storage

The Hieff Clone™ Universal One Step Cloning Kit should be stored at -25°C~ -15°C for 1 year.

Instructions

1. Calculation of the amount of vectors and fragments

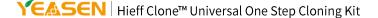
The total insertion volume of the optimal fragment and carrier in the recombinant reaction system is 0.02-1 pmol, 1-3 fragment is 0.02-0.5 pmol, and 4-6 fragment is 0.5-1 pmol. The optimum molar ratio of vector to insert fragment was 1:3. The corresponding DNA quality can be calculated by the following formula:

Linearized vector usage ng = (insert base logarithm \times 0.65 \times vectors and fragments pmol) / (1+3n)

Insert fragment usage ng = (insert base logarithm \times 0.65 \times vectors and fragments pmol \times 3) / (1+3n)

N represents the number of inserted fragments

2. Recombination reaction system (It is recommended to prepare on ice. All components should be mixed well before





use)

Component	1~3 fragments	4~6 fragments	Negative control
ddH ₂ O	Up to 20 μL	Up to 20 μL	Up to 20 μL
Hieff Clone™ Universal One Step Cloning Kit	10 μL	10 μL	10 μL
Total fragments	0.02-0.5 p mol	0.5 - 1 p mol	ΧμL

- 3. Recombination reaction conditions
- 3.1 After the preparation of the system, gently suck and beat the components with a pipette, mix them evenly, and collect the reaction solution to the bottom of the tube by brief centrifugation.
- 3.2 When one fragment is inserted and the total amount of DNA is less than 300 ng, the recommended reaction condition is 50 °C for 5 min; When the number of inserted fragments is 2-4, the recommended reaction condition is 50 °C, 15 min; When the number of inserted fragments is 5-6, the recommended reaction condition is 50 °C for 30 min. It is recommended that the reaction be carried out on instruments with accurate temperature control such as PCR instrument or water bath.
- 3.3 The reaction products can be transformed directly or stored at -20 °C and thawed and transformed when necessary.

Notes

- 1. Please wear the necessary PPE, such lab coat and gloves, to ensure your health and safety.
- 2. For research use only.