



Ver. HB230322

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 × 0.65 × vectors and fragments pmol) / (1+3n)

Insert fragment usage ng = (insert base logarithm × 0.65 × vectors and fragments pmol × 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	X μL

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.