Ver. HB221108

# 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) |
|---|---|---|
| 10922-A | Hieff Clone™ Universal Enzyme Premix | 200 μL |
| 10922-B | 500 bp Control Insert (25 ng/μL) | 5 μL |
| 10922-C | pUC 19 Control Vector, linearized (50 ng/μL) | 5 μL |

## Specifications

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

## Shipping and Storage

The Hieff Clone™ Universal One Step Cloning Kit is shipped with dry ice; and can be stored at-35°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 |
|---|---|---|---|
| dd$H_2O$ | 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.