Single Cell WGA Kit

Single Cell Whole Genome Amplification Kit

Catalog Number:

S665725 (24 rxns)
S665725 (96 rxns)

Storage conditions: Please send the kit on dry ice and store all components in a refrigerator at -20°C for up to 12 months immediately after receipt. For longer term storage, please store below -70℃.

Products content

Component	24 rxns	96 rxns
Cell Lysis Buffer	240 μl	960 μl
Cell Lysis Enzyme	16 μl	64 μl
Pre-Amp Buffer	120 μl	480 μl
Pre-Amp Enzyme Mix	7 μl	28 μl
Amplification Buffer	1.5 ml	4 x 1.5 ml
Amp Enzyme Mix	50 μl	200 μl

Products Introduction

The Single Cell Whole Genome Amplification Kit can be used as a template for whole genome amplification of single cells or micro samples. The total time for single-cell amplification is about 3 hours, and 2-5 μg of genomic DNA, with a size of 200-1500 bp, can be obtained after lysis, pre-amplification and amplification. The amplified product can be widely used in second-generation sequencing, large fragment copy number variation analysis, SNP typing, qPCR analysis and gene chip analysis.

Bring your own instruments and reagents

PCR instrument

Reaction tubes: low adsorption tubes recommended

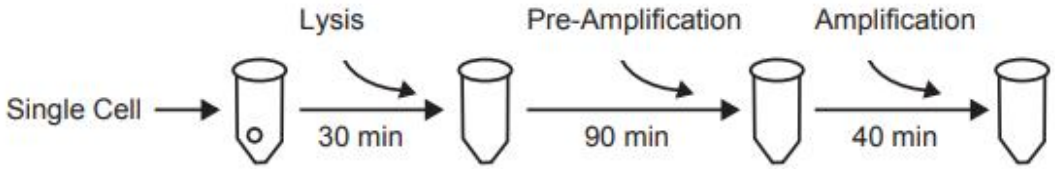
Gun Heads: High quality filtered gun heads are recommended

Microcentrifuge, vortex mixer

caveat

The sensitivity of this product is very high, the experimental operation should be completed in a positive pressure ultra-clean bench or clean environment, the concentration of the amplification reaction products is high, should be well isolated to avoid aerosol contamination caused by amplification products.

Operation flow diagram



procedure

Pre-experiment preparation

Single cells were obtained by flow cytometry sorting, buffer dilution, micromanipulation and laser microdissection. It is recommended that the cells be washed prior to the experiments with a 1× PBS solution free of Mg2+ and Ca2+ , taking care to ensure that the volume of PBS solution in subsequent experiments does not exceed 2 μl.

take note of

Since the whole experiment is carried out in the same PCR tube and the reaction volume is small, the pipette tip should not touch the liquid in the tube when adding liquid, so as to avoid taking single cells or DNA out of the reaction system; when pipetting, please add the liquid along the wall of the tube carefully and do not blow the liquid in the PCR tube; before the reaction, please centrifuge briefly to make sure that the liquid in the reaction system is mixed evenly. Thaw the cell lysate, pre-amplifier and amplifier on ice before use.

cell lysis

1）Mix Cell Lysis Buffer and Cell Lysis Enzyme according to the number of reactions N, shake to mix, centrifuge briefly and set aside.

Cell Lysis Mix	volumetric
Cell Lysis Buffer	9.4 μl × N
Cell Lysis Enzyme	0.6 μl × N
Total Volume	10 μl × N

2）Mix single cells with the cell lysis mix in a PCR tube and run the following program.

cycle number (math.)	temp	timing
1	50°C	20 min
1	95°C	10 min
1	4°C	Hold

2. Pre-amplification reaction

1）Mix Cell Lysis Buffer and Cell Lysis Enzyme according to the number of reactions N, shake to mix, centrifuge briefly and set aside.

Pre-amplification mix	volumetric
Pre-Amp Buffer	4.75 μl × N
Pre-Amp Enzyme Mix	0.25 μl × N
Total Volume	5 μl × N

2）Add 5 μl of pre-amplification mix to 10 μl of lysis reaction product from the previous step and run the following program.

cycle number (math.)	temp	timing
1	95°C	2 min
12	95°C	15 s
12	15°C	50 s
12	25°C	40 s
12	35°C	30 s
12	65°C	40 s
12	75°C	40 s
1	4°C	Hold

3. Amplification reaction

1）Mix Amplification Buffer and Amp Enzyme Mix according to the number of reactions N, mix with shaking, centrifuge briefly and set aside.

Amplification Mix	volumetric
Amplification Buffer	58 μl × N
Amp Enzyme Mix	2 μl × N
Total Volume	60 μl × N

2）Add 60 μl of amplification mix to 15 μl of pre-amplification reaction product from the previous step and run the following program.

cycle number (math.)	temp	timing
1	95°C	2 min
14	95°C	15 s
14	65°C	1 min
14	75°C	1 min
1	4°C	Hold

Note: The number of cycles can be adjusted as needed, 14 cycles are recommended for single cells obtained by flow sorting, etc.

Amplification product detection

1. Agarose gel electrophoresis

5 μl of the amplified product was subjected to agarose gel electrophoresis (1% agarose gel, 110 V, 25-35 min), and the amplified product was 200-1500 bp in size.

2. Quantitative

Amplification products were subjected to magnetic bead or column purification, and purified products were quantified using Qubit with a final yield of 2-5 μg.