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Single Cell WGA Kit

Product Number: PCK244

Shipping and Storage

Please send in dry ice and immediately store all components in a -20℃ constant temperature refrigerator after receiving the reagent kit, which can be stored for 6 months. If you need to store it for a longer period of time, please store it below -70℃.

Components

Component	PCK244 24rxns	PCK244 96rxns
Cell Lysis Buffer	240μl	960μl
Cell Lysis Enzyme	16μl	64μl
Pre-Amp Buffer	120μl	480μl
Pre-Amp Enzyme Mix	7μl	28μl
Amplification Buffer	1.5ml	4×1.5ml
Amp Enzyme Mix	50μl	200μl

Description

The single-cell whole genome amplification kit can achieve whole genome amplification using single cells or trace samples as templates. The single-cell amplification reaction time is short, with a total process of about 3 hours. After lysis, pre amplification, and amplification, 2-5μg of genomic DNA can be obtained, with a size of around 200-1500bp. The amplified products can be widely used for second-generation sequencing, large segment copy number variation analysis, SNP typing, qPCR analysis, gene chip analysis, etc.

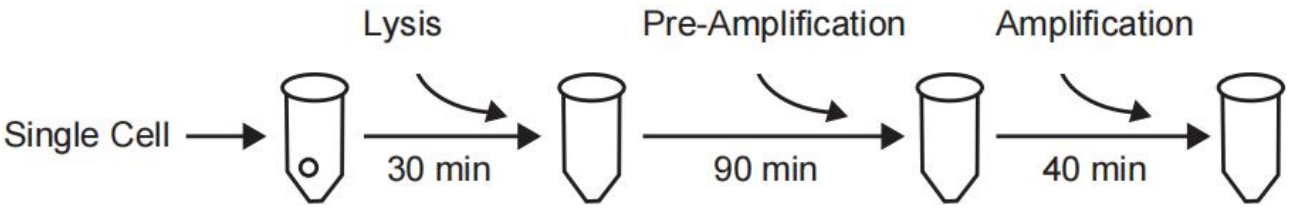
Self provided instruments and reagents

1. PCR instrument
2. Reaction tube: It is recommended to use a low adsorption tube
3. Gun head: It is recommended to use high-quality filtering gun head
4. Micro centrifuge, vortex mixer

Note

This product has extremely high detection sensitivity, and experimental operations should be completed in a positive pressure ultra clean workbench or clean environment. The concentration of amplification reaction products is high, and isolation should be done to avoid aerosol pollution caused by amplification products.

Operation process diagram



Protocol

1. Preparation before the experiment

Single cells were obtained through flow cytometry sorting, buffer dilution, micromanipulation, and laser microdissection. It is recommended to clean the cells before the experiment. The cleaning solution is a 1×PBS solution free of $Mg^{2+}$and Ca^{2+}, and

attention should be paid to ensuring that the volume of PBS solution in subsequent experiments does not exceed 2µl.

Note: Due to the fact that the entire experiment was conducted in the same PCR tube and the reaction volume was small, the pipette tip should not come into contact with the liquid in the tube during the liquid addition operation to avoid bringing single cells or DNA out of the reaction system; When transferring the liquid, please add it carefully along the tube wall and do not blow the liquid in the PCR tube; Before the reaction, please perform a brief centrifugation to ensure that the liquids in the reaction system are evenly mixed.

Before use, please thaw the cell lysis enzyme, pre amplification enzyme, and amplification enzyme on ice.

2. Cell lysis

2.1. According to the number of reactions N, mix the Cell Lysis Buffer and Cell Lysis Enzyme, shake well, and centrifuge briefly for later use.

Cell lysis mixture	Volume
Cell Lysis Buffer	9.4µl × N
Cell Lysis Enzyme	0.6µl × N
Total Volume	10µl × N

2.2. Mix the mixture of single cell and cell lysis in a PCR tube and run the following procedure.

Cycles	Temperature	Time
	50℃	20 min
1	95℃	10 min
	4℃	Hold

3. Preamplification

3.1. According to the number of reactions N, mix Pre Amp Buffer and Pre Amp Enzyme Mix, shake well, and centrifuge briefly for later use.

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

3.2. Add 5µl of pre amplification mixture to the 10µl cleavage reaction product in the previous step, and run the following program.

Cycles	Temperature	Time
1	95℃	2 min
	95℃	15 s
	15℃	50 s
12	25℃	40 s
	35℃	30 s
	65℃	40 s
1	4℃	Hold

4. Amplification reaction

According to the number of reactions N, mix the Amplification Buffer and Amp Enzyme Mix, shake well, and centrifuge briefly for later use.

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

4.1. Add 60ul of amplification mixture to the 15µl pre amplification reaction product in the previous step, and run the following program.

Cycles	Temperature	Time
1	95℃	2 min
14	95℃	15 s
	65℃	1 min
	75℃	1 min
1	4℃	Hold

Explanation: The number of cycles can be adjusted as needed. For single cells obtained through flow sorting and other methods, it is recommended to have 14 cycles.

Amplification product detection

1. Agarose gel electrophoresis

   Take 5μl amplification product for agarose gel electrophoresis (1% agarose gel, 110V, 25-35 minutes), and the size of amplification product is 200-1500bp.

2. Ration

   Magnetic bead or column purification was performed on the amplified product, and the purified product was quantified using Qubit. The final yield was 2-5μg.