Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Single Cell WGA Kit

Product Number: PCK44

Shipping and Storage

Please ship in dry ice and immediately store all components in a -20℃ constant temperature refrigerator after receiving the reagent kit, which can be stored for 12 months. For longer term storage, please store below -70℃.

Components

Component	PCK44 24rxns	PCK44 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

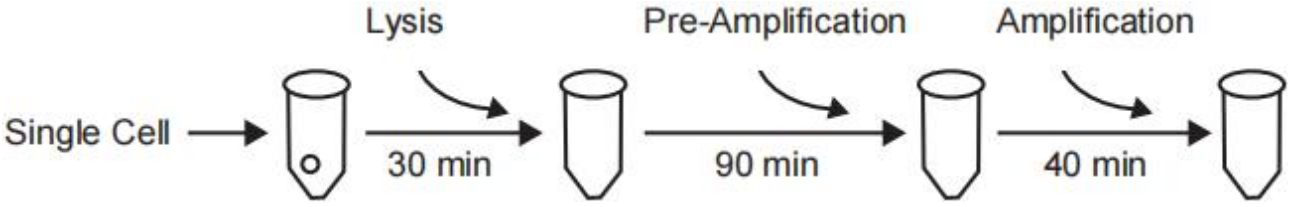
Description

The Single Cell WGA 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 consumables

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 filter gun head
4. Micro centrifuge, vortex mixer

Schematic diagram of operation process



Note

This product has extremely high detection sensitivity, and experimental operations should be completed on 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.

Protocol

1. Preparation before 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, and the cleaning solution 1× PBS solution should be without $Mg^{2+}$and Ca^{2+} ,

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

2. Note

Due to the fact that the entire experiment was conducted in the same PCR tube and the reaction volume was small, the pipette suction head should not come into contact with the liquid in the tube during the liquid addition operation to avoid damaging single cells or DNA out of the reaction system; When transferring 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 liquid in the reaction system is evenly mixed.

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

3. Cell lysis

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

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

3.2. Mix the mixture of single cell and cell lysis into a PCR tube and run the following program.

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

4. Pre amplification reaction

4.1. 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	4.75 μl × N
Pre-Amp Enzyme Mix	0.25 μl × N
Total Volume	5 μl × N

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

Cycles	Temperature	Time
1	95℃	2 min
12	95℃	15 s
	15℃	50 s
	25℃	40 s
	35℃	30 s
	65℃	40 s
	75℃	40 s
1	4℃	Hold

5. Amplification reaction

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

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

5.2. Add 60μl of amplification mixture to 15μl of 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

Note:The number of cycles can be adjusted as needed, and 14 cycles are recommended for single cells obtained through flow sorting and other methods.

Amplification product detection

1. Agarose gel electrophoresis

Take 5μL The amplified product was subjected to agarose gel electrophoresis (1% agarose gel, 110V, 25-35 minutes), and the size of the amplified product was 200-1500bp.

2. Quantitative

Perform magnetic bead or column purification on the amplified product, and quantify the purified product using Qubit. The final yield is 2-5μg.