Version: 02/2024

Single Cell WGA Kit (MDA)

Cat. No. : CW2843S (24 rxns)
CW2843M (96 rxns)

Storage Condition: Ship with dry ice, and store all components in a -20 ℃ refrigerator immediately after receiving the kit. It can be stored for 6 months. For long-term storage, please store below -70 ℃.

Components

Component	CW2843S (24 rxns)	CW2843M (96 rxns)
SC-DNA Polymerase	48 μL	192 μL
SC-Reaction Buffer	1 mL	4×1 mL
Buffer D	1 mL	1.5 mL
Buffer N	1 mL	1.5 mL
DTT, 1 M	1 mL	1 mL
PBS	1 mL	1.5 mL

Introduction

The single-cell whole-genome amplification kit is based on the isothermal amplification system of MDA, which can achieve whole-genome amplification with a single cell or micro sample as a template. After the whole genome of a single cell is amplified, the size of the amplified product is 2-100 kb. It can be widely used in next-generation sequencing, large fragment CNV analysis, microsatellite analysis, qPCR analysis, gene chip analysis, etc.

The Phi29 DNA polymerase used in this kit is a DNA polymerase cloned from bacteriophage. It has strong strand displacement activity and chain affinity. A single polymerization reaction can achieve continuous polymerization extension of up to 100 kb, and its amplified product is suitable for a variety of downstream applications. Phi29 DNA polymerase also has strong 3'-5' exonuclease activity, ensuring high fidelity in DNA synthesis. A single reaction normally yields greater than 20 μg of genomic DNA with high coverage.

Genome as amplification template

This protocol is suitable for indiscriminate amplification of the whole genome using more than 1 ng of purified genomic DNA as a template. If the genome integrity and purity are high enough, less starting DNA can also be used.

1. Prepare Buffer D1 and N1 (the volume given in the table below is enough for 12 reactions, and it can be stored at -20 ℃ if it is not completely used up in one experiment, but the storage time cannot exceed 3 months).

Component	Buffer D1	Buffer N1
Buffer D	7 μL	—
Buffer N	—	9 μL
Water	25 μL	51 μL
Total Volume	32 μL	60 μL

2. Add 2.5 μL DNA sample to PCR tube. If the sample volume is less than 2.5 μL, fill to 2.5 μL with water or TE.
3. Add 2.5 μL Buffer D1, flick the tube wall to mix and centrifuge briefly.
4. Incubate at room temperature (15-25 ℃) for 3 min.
5. Add 5 μL Buffer N1, flick the tube wall to mix and centrifuge briefly. Keep samples on ice before the following reaction is ready.
6. Prepare the reaction mixture according to the table below, mix well and centrifuge briefly.

Component	Volume
SC-Reaction Buffer	38 μL
SC-DNA Polymerase	2 μL
Total Volume	40 μL

7. Immediately add 40 μL of the reaction mixture to the prepared 10 μL DNA sample (step 5), mix by flicking the tube wall and centrifuge briefly to collect.
8. Incubate at 30 ℃ for 2 h. The incubation time can be extended to increase the yield if necessary.
9. Incubate at 65 ℃ for 5 min to inactivate SC-DNA Polymerase.

Note: The amplified product is high-concentration genomic DNA, please use water or TE to dilute to an appropriate concentration for downstream experiments. The amplified product can be widely used in whole genome and exome sequencing, qPCR analysis, gene chip analysis, etc.

Reagents to be Supplied by User

Centrifuge

Water bath or PCR machine

Reaction tubes: low adsorption PCR tubes are recommended

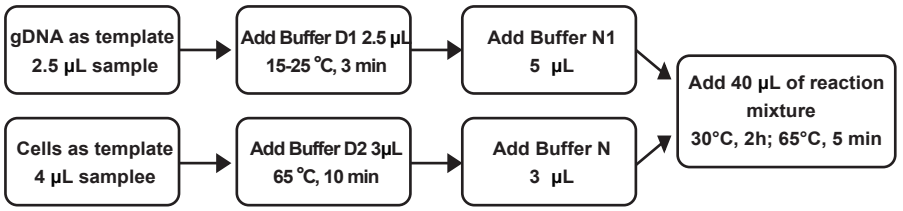
Tip: High quality filter tips are recommended to prevent contamination

Deionized water

Notes

1. The detection sensitivity of this product is extremely high. The experimental operation should be completed in a cleanbench. The concentration of the amplification product is high, and isolation should be done to avoid aerosol pollution caused by the amplification product.
2. Using a low-quality sample as template will affect the quality of the final amplification product. The use of a large amount of degraded or fragmented DNA as starting sample should be avoided.

Schematic Diagram of the Operation Process



Procedure

Cells as amplification template

This protocol is suitable for indiscriminate amplification of the whole genome using 1-1000 cells as templates. Freshly prepared cell samples should be used to ensure the integrity of the starting genome. Do not use cells that have undergone apoptosis.

1. Prepare Buffer D2 (the volume of Buffer D2 given in the table below is enough for 12 reactions, and it can be stored at -20 ℃ if it is not completely used up in one experiment, but the storage time cannot exceed 3 months).

Component	Volume
Buffer D	33 μL
DTT, 1 M	3 μL
Total Volume	36 μL

2. Add 4 μL of cell samples (resuspended in PBS) to PCR tubes. If the sample volume is less than 4 μL, fill to 4 μL with PBS.
3. Add 3 μL Buffer D2, flick the tube wall to mix and centrifuge briefly to collect. Make sure that the cells are not sticking to the tube wall. Do not use a pipette to prevent the cell sample from sticking to the pipette tip.
4. Incubate the sample at 65 ℃ for 10 min.
5. Add 3 μL of Buffer N, mix by flicking the tube wall and centrifuge briefly. Keep samples on ice before the following reaction is ready.
6. Prepare the reaction mixture according to the table below, mix well and centrifuge briefly.

Component	Volume
SC-Reaction Buffer	38 μL
SC-DNA Polymerase	2 μL
Total Volume	40 μL

7. Immediately add 40 μL of the reaction mixture to the prepared 10 μL DNA sample (step 5), mix by flicking the tube wall and centrifuge briefly to collect.
8. Incubate at 30 ℃ for 2 h. The incubation time can be extended to increase the yield if necessary.
9. Incubate at 65 ℃ for 5 min to inactivate SC-DNA Polymerase.

   Note: The amplified product is high-concentration genomic DNA, please use water or TE to dilute to an appropriate concentration for downstream experiments. The amplified product can be widely used in whole genome and exome sequencing, qPCR analysis, gene chip analysis, etc.