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Single Cell WGA Kit(MDA)

Product Number: PCK243

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	PCK243 24rxns	PCK243 96rxns
SC-DNA Polymerase	48μl	192μl
SC-Reaction Buffer	1ml	4×1ml
Buffer D	1ml	1.5ml
Buffer N	1ml	1.5ml
DTT, 1M	1ml	1ml
PBS	1ml	1.5ml

Description

The single-cell whole genome amplification kit is based on an MDA isothermal amplification system, which can achieve whole genome amplification using single cells or trace samples as templates. The amplification product size of a single-cell whole genome after amplification is between 2-100 kb, which can be widely used for second-generation sequencing, large segment copy number variation analysis, microsatellite analysis, qPCR analysis, gene chip analysis, etc.

The Phi29 DNA polymerase used in this kit is a DNA polymerase cloned from bacteriophages, which has strong chain displacement activity and affinity. A single polymerization reaction can achieve continuous polymerization extension of up to 100 kb. Its amplification products are suitable for various downstream applications. Phi29 DNA polymerase also has strong 3 '-5' exonuclease activity, ensuring high fidelity of DNA synthesis. Under normal circumstances, a reaction can produce genomic DNA with a high coverage greater than 20μg.

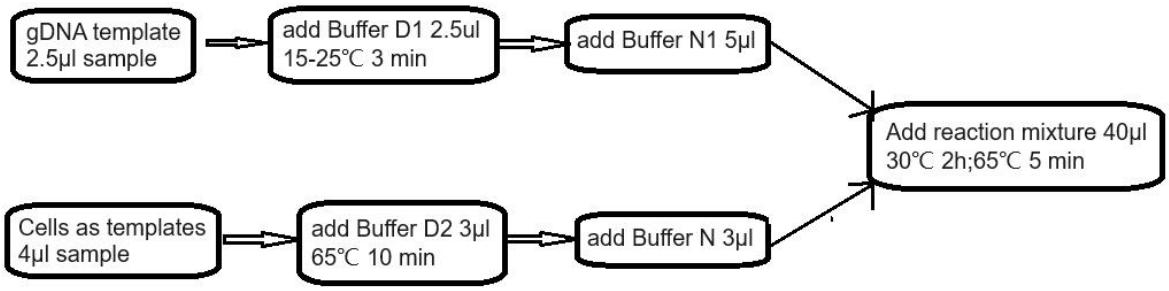
Self provided instruments and reagents

1. Centrifuge
2. Water bath or PCR instrument
3. Reaction tube: It is recommended to use a low adsorption PCR tube
4. Gun tip: It is recommended to use high-quality filtering gun tips to prevent contamination
5. Deionized water

Note

1. This product has extremely high detection sensitivity, and the experimental operation should be completed in a positive pressure ultra clean workbench. The concentration of amplification reaction products is high, and isolation should be done to avoid aerosol pollution caused by amplification products.
2. Using low-quality samples as templates can affect the quality of the final amplification product, and it is advisable to avoid using a large amount of degraded and fragmented DNA as starting samples.

Operation process diagram



Protocol

1. Cell template amplification

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

1.1. Prepare Buffer D2 (the volume of Buffer D2 given in the table below is sufficient for 12 reactions. If it is not completely used up in one experiment, it can be stored at -20°C, but the storage time cannot exceed 3 months).

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

1.2. Add 4μl of cell sample (resuspended in PBS) to the PCR tube. If the sample volume is less than 4μl, please use PBS to make up to 4μl.

1.3. Add 3μl Buffer D2, mix lightly with the tube wall, and collect briefly by centrifugation. Please ensure that the cells do not adhere to the tube wall, and do not use a pipette to blow to prevent the cell sample from adhering to the suction head of the pipette.

1.4. Incubate the sample at 65℃ for 10 minutes.

1.5. Add 3μl Buffer N, mix lightly on the tube wall and centrifuge briefly. Please place the sample on ice before preparing for the next reaction.

1.6. Prepare the reaction mixture according to the table below, mix well and centrifuge briefly.

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

1.7. Immediately add 40μl of reaction mixture to the prepared 10μl DNA sample (step 5), gently flick the tube wall and mix well, then centrifuge briefly for collection.

1.8. Incubate at 30℃ for 2 hours, and if necessary, extend the incubation time to increase yield.

1.9. Incubate at 65℃ for 5 minutes to inactivate SC-DNA Polymerase.

Note: The amplified product is high concentration genomic DNA. Please dilute it to an appropriate concentration with water or TE before conducting downstream experiments. The amplified products can be widely used in whole genome and exon sequencing, qPCR analysis, gene chip analysis, and so on.

2. Genome as template amplification

This scheme is suitable for undifferentiated amplification of the entire genome using genomic DNA with a purity greater than 1ng as a template. If the genome integrity and purity are high enough, even fewer starting DNA can be used.

2.1. Prepare Buffer D1 and N1 (the volume given in the table is sufficient for 12 reactions. If not completely used up in one experiment, it can be stored at -20°C, but the storage time cannot exceed 3 months).

Component	Buffer D1	Buffer N1
Buffer D	7μl	-

Buffer N	-	9μl
H_2O	25μl	51μl
Total	32μl	60μl

2.2. Add 2.5μl of DNA sample to the PCR tube. If the sample volume is less than 2.5μl, please use water or TE to make up to 2.5μl.

2.3. Add 2.5μl Buffer D1, mix lightly with the tube wall and centrifuge briefly.

2.4. Incubate at room temperature (15-25℃) for 3 minutes.

2.5. Add 5μl Buffer N1, mix lightly with the tube wall and centrifuge briefly. Please place the sample on ice before preparing the next reaction.

2.6. Prepare the reaction mixture according to the table below, mix well and centrifuge briefly.

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

2.7. Immediately add 40μl of reaction mixture to the prepared 10μl DNA sample (step 5), gently flick the tube wall and mix well, then centrifuge briefly for collection.

2.8. Incubate at 30℃ for 2 hours, and if necessary, extend the incubation time to increase yield.

2.9. Incubate at 65℃ for 5 minutes to inactivate SC-DNA Polymerase.

Note: The amplified product is high concentration genomic DNA. Please dilute it to an appropriate concentration with water or TE before conducting downstream experiments. The amplified products can be widely used in whole genome and exon sequencing, qPCR analysis, gene chip analysis, and so on.