Discover-sc
Single Cell WGA Kit

N603

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Instruction for Use

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Vazyme

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01/Product Description

Discover-sc Single Cell WGA Kit is based on multiple displacement amplification (MDA) and is designed for unbiased whole genome amplification from single cell and other micro samples. The sizes of amplification products are between 2 kb to 100 kb, with a coverage greater than 95% and an average length higher than 15 kb. The products can be widely applied to whole genome sequencing, whole exome sequencing, large fragment copy number variation analysis, microsatellite analysis, qPCR analysis, or gene chip analysis.

The Phi29 DNA polymerase used in this kit is cloned from phage and has a strong strand displacement activity. Melting and replication of the DNA chain with complex structure can be achieved. Meanwhile, Phi29 has a strong chain affinity and can achieve up to 100 kb of continuous polymerization, and the amplification product can be applied to almost all of the downstream genome analysis. Phi29 DNA polymerase also possesses a potent 3'→5 'exonuclease (proofreading) activity, its fidelity is 1,000-fold that of Taq enzyme, thus ensuring the high fidelity of DNA replication.

All the reagents provided in the kit have undergone rigorous quality control and functional testing to ensure the optimal stability and repeatability.

02/Components

Components	N603-01 (24 rxns)	N603-02 (96 rxns)
Discover-sc WGA Enzyme Mix	48 μl	192 μl
Discover-sc WGA Master Buffer	750 μl	3 × 1 ml
Buffer D	1 ml	2 × 1 ml
Stop Solution	1 ml	2 × 1 ml
DTT, 1 M	1 ml	1 ml
Cell Storage Buffer	1 ml	2 × 1 ml
Nuclease-free ddH_2O	1 ml	2 × 1 ml

▲ The color marked in the product components table represents the color of each tube lid.

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃. For long-term storage, please store at -85 ~ -65℃.

04/Applications

Discover-sc Single Cell WGA Kit can be used for unbiased whole genome amplification from single cells and other micro samples. It is not suitable for low-quality samples such as fixed cells and FFPE sample.

Human and animal

- ◇ Stem cell research
- ◇ Research on tumor progression
- ◇ Cancer stem cell analysis
- ◇ Genetic engineering animals genotyping
- ◇ Chimeras study
- ◇ Preimplantation genetic screening
- ◇ Circulating fetal cells analysis
- ◇ Transgenic animals genotyping
- ◇ SNP, CNVs and other biomarkers study

Bacteria

- ◇ Pathogen analysis
- ◇ Metagenomics research
- ◇ Microbial typing

Plant

- ◇ Pollen analysis

05/Self-prepared Materials

- ◇ DNA evaluation:
  Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121).
- ◇ Purification beads:
  VAHTS DNA Clean Beads (Vazyme #N411) or Agencourt AMPure XP Reagent (Beckman #A63880/A63881/A63882).
- ◇ Other materials:
  Centrifuge, microscope, low binding EP tube, RNase-free PCR tube, water bath or thermal cycler and qPCR instrument.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. The detection sensitivity of this product is extremely high, and the experimental operation should be completed in a positive pressure ultra-clean workbench. Do not use it with common PCR operating platforms to avoid interference of samples by external factors or DNA contamination.
2. All the components in this product should be stored in an environment free from nucleic acid pollution to avoid reagent contamination.
3. The cell lysis method in this product cannot effectively lyse the cell wall. Eukaryotic cells with cell walls need to be lysed after removing the cell wall, or use purified DNA for reaction. Mammalian cells can be lysed and amplified directly using this protocol.
4. Using a low-quality sample as a template will affect the quality of the final amplified product. Try to avoid using a large amount of degraded and fragmented DNA as the template.
5. Set up positive and negative control to verify whether the system is working properly.
6. The concentration of the whole genome obtained from a single amplification reaction is relatively high. Try to avoid bringing the product to the public experimental area to prevent aerosol pollution to other experiments.
7. All the components in the kit have been optimized, please do not change the reaction system during use.

07/Sample Preparation

Cell culture media or other components in the sample may inhibit the reaction. Please minimize unnecessary sample volume to reduce the possible impact on the reaction system.

07-1/Cells Sample Preparation

Cell numbers: This product can directly use 1 - 1,000 cells as the starting sample for whole genome amplification, and too many cells will inhibit the reaction.

Cell acquisition method: Since the integrity of the genome directly affects the integrity of the amplified product, please determine the effect of the cell acquisition method on the cell viability. It is recommend that the cell viability should be identified after cell sample is collected. The genome of dead cells may be degraded, leading to the failure of the experiment. It is recommended to amplify immediately after the identification is qualified. Improper storage conditions will cause the genome degradation.

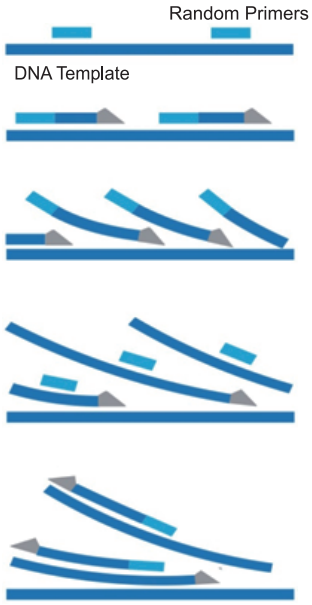
Storage: If you need to store for a certain period of time, separate the single cells into 4 μl of Cell Storage Buffer, and then store the sample at -85 ~ -65℃ or lower temperature. After taking it out, please proceed to the subsequent reaction immediately. The separated living cells can also be stored in cell cryopreservation solution. Resuscitate the cells when they need to be used. Please confirm whether the cells are alive after resuscitation using this storage solution. The dead cells will have obvious DNA fragmentation and cause the reaction to fail.

Cell culture medium test: For cultured cells, please confirm if the cell culture medium has inhibitory effects on the experiment. The medium that is expected to be carried by the cell sample can be added to a control genomic reaction to test whether the medium inhibits the amplification reaction. If the effect of the medium on the reaction cannot be confirmed, resuspend the cells in PBS before lysing and reacting.

07-2/DNA Sample Preparation

Try to avoid contamination and ensure the integrity of the genome. Degraded and fragmented DNA will cause the experiment to fail.

08/Mechanism & Workflow



Random primers bind to multiple sites of the DNA template.

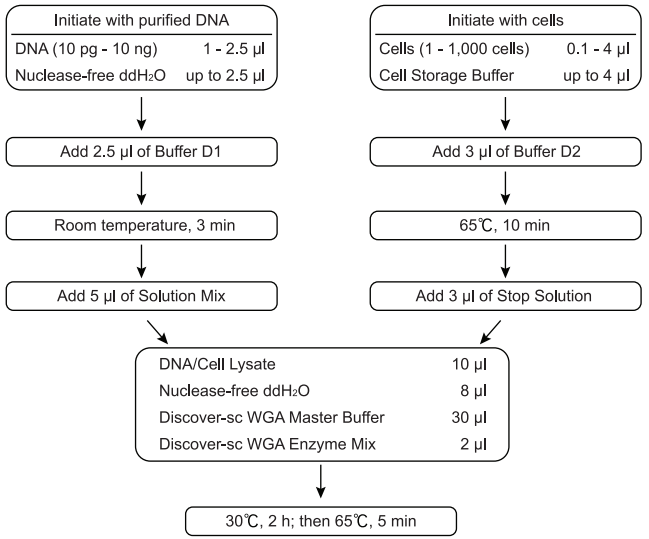
Phi29 DNA polymerase simultaneously initiates DNA replication at multiple primer binding sites.

Chain synthesis reaction in process replaces encountered DNA chain in synthesis and continue elongation, and produces replaced single strand DNA.

Replaced single strand DNA is bound by random primers as templates.

Random primers initiate new DNA synthesis and chain replacement reaction, to synthesize double strand DNA of high molecular size.

Schematic diagram of MDA mediated with Phi29 DNA polymerase



Schematic Diagram of Operating Process

09/Experiment Process

09-1/Amplify Genomic DNA From Single Cell

This scheme is suitable for 1 - 1,000 initial cells. Please use the freshly prepared cell samples to ensure the integrity of initiated genome and do not use apoptotic cells.

1. Prepare Buffer D2. (The Buffer D2 volume in the following table is sufficient for 12 reactions. Store the buffer at -30 ~ -15℃ after the experiment, and use up within 3 months.)

Components	Volume
DTT, 1 M	4 µl
Buffer D	36 µl
Total	40 µl

2. Add 4 µl of cell samples (resuspended in Cell Storage Buffer) to a PCR tube. Please make up to 4 µl with Cell Storage Buffer if the sample volume is less than 4 µl.

3. Add 3 µl of Buffer D2. Flick the tube wall to mix the cells followed by brief centrifugation.

▲ Please make sure that the cells are not attached to the tube wall. DO NOT mix the cells with pipettors to avoid that the cells are attached to the tips.

4. Incubate at 65℃ for 10 min.

5. Add 3 µl of Stop Solution, and mix by flick the tube wall followed by brief centrifugation. Place the sample on the ice before the next reaction system is prepared.

6. Prepare the mixture of reaction as follows:

Components	Volume
Nuclease-free ddH$_2$O	8 µl
Discover-sc WGA Master Buffer	30 µl
Discover-sc WGA Enzyme Mix	2 µl
Total	40 µl

▲ Please add the above components in order. Mix by vortex and brief centrifugation before adding Nuclease-free ddH$_2$O and Discover-sc WGA Master Buffer. Please proceed to next step immediately after adding Discover-sc WGA Enzyme Mix.

7. Immediately add 40 µl of reaction mixture to the prepared 10 µl of DNA sample (prepared in Step 5). Mix by flick the tube wall followed by brief centrifugation.

8. Incubate at 30℃ for 2 h.

9. Incubate at 65℃ for 5 min to inactivate the Discover-sc WGA Enzyme Mix.

10. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with Nuclease-free ddH$_2$O or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, Array CGH, etc.

09-2/Amplify Purified Genomic DNA

This scheme is suitable for the whole genome amplification from purified genomic DNA with amount >10 ng. Less Input DNA (1 - 10 ng of eukaryotic genome DNA, 10 - 100 pg of bacterial genome DNA) can also be used if the integrity and purification of genome is high enough.

1. Prepare Buffer D1 and Stop Solution Mix. (The Buffer D1 and Stop Solution Mix volume in the following table are sufficient for 12 reactions. Store the buffer at -30 ~ -15℃ after the experiment, and use up within 3 months.)

Prepare Buffer D1

Components	Volume
Buffer D	7 μl
Nuclease-free ddH_2O	25 μl
Total	32 μl

Prepare Stop Solution Mix

Components	Volume
Stop Solution	9 μl
Nuclease-free ddH_2O	51 μl
Total	60 μl

2. Add 2.5 μl of DNA samples to a PCR tube. If the sample volume is lower than 2.5 μl, please make up to 2.5 μl with Nuclease-free ddH_2O or TE.

3. Add 2.5 μl of Buffer D1. Flick the tube wall to mix thoroughly followed by brief centrifugation.

▲ DO NOT mix the cells with pipettors to avoid that the integrity is affected and the cells are attached to the tips.

4. Incubate at room temperature for 3 min.

5. Add 5 μl of Stop Solution Mix. Flick the tube wall to mix thoroughly followed by brief centrifu gation. Place the sample on the ice before the next step.

6. Prepare the mixture of reaction.

Components	Volume
Nuclease-free ddH_2O	8 μl
Discover-sc WGA Master Buffer	30 μl
Discover-sc WGA Enzyme Mix	2 μl
Total	40 μl

▲ Please add the above components in order. Mix by vortex and brief centrifugation after adding Nuclease-free ddH_2O and Discover-sc WGA Master Buffer. Please proceed to next step immediately after adding Discover-sc WGA Enzyme Mix.

7. Immediately add 40 μl of reaction mixture to 10 μl of DNA sample (prepared in Step 5), flick the tube wall to mix thoroughly followed by brief centrifugation.

8. Incubate at 30℃ for 2 h.

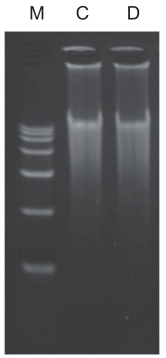
9. Incubate at 65℃ for 5 min to inactivate the Discover-sc WGA Enzyme Mix.

10. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with Nuclease-free ddH_2O or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, Array CGH, etc.

10/Amplification Products Analysis

Electrophoresis Analysis

The sizes of amplification products using Discover-sc Single Cell WGA Kit are between 2 kb to 100 kb, with an average length more than 15 kb.



M: DNA marker (DL15,000)

C: Amplification products of single cells

D: Amplification products of genome

Fig 1. Electrophoresis analysis of amplification products

11/FAQ & Troubleshooting

◇ No amplified products:

① **Sample loss during collection:**

Re-collect the sample. Avoid sample loss caused by incorrect operation.

② **Genome DNA samples contain components that inhibit the reaction:**

Purify or dilute DNA samples. If there is ethanol precipitation or rinsing process during the purification of samples, the residual ethanol can inhibit the reaction. Please make sure there are no ethanol residue.

③ **High Reaction Temperature:**

The reaction temperature should be controlled at 30℃. Over high temperature can inactivate the enzyme. If use PCR instrument with hot-lid, please set the temperature of hot-lid at 70℃.

◇ 7.5 - 20 μg of DNA is amplified, but chromosomal malposition or allele loss is detected:

① **Using genomic DNA as initial template:**

The genomic DNA may be degraded, please use intact DNA or more amount of DNA as templates.

② **Using cells as initial template:**

DNA degradation was caused by apoptosis or cell fixation. Cells with cell walls (e.g. plant cells) that are hard to lyse are not suitable for direct use as starting materials.

◇ There are amplification products in the negative control but the downstream test results are negative (e.g. qPCR):

The high molecular weight DNA products were amplified by the random annealing of primers in the negative control reactions, but the products do not affect downstream analysis of the target products.

◇ 7.5 - 20 μg of DNA was amplified in negative control and the downstream test results are positive (e.g. qPCR):

The reaction is contaminated. Since the reaction is very sensitive to micro amount of DNA, please replace all the reagents and supplies that may be contaminated with exogenous DNA.