Product Information

GenomePlex® Single Cell
Whole Genome Amplification Kit

Product Number WGA4
Storage Temperature −20 °C

TECHNICAL BULLETIN

Product Description
GenomePlex is a Whole Genome Amplification (WGA) method that allows the researcher to generate a representative amplification of genomic DNA. The kit utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR-amplifiable OmniPlex® Library molecules flanked by universal priming sites. The OmniPlex library is then PCR amplified using universal oligonucleotide primers and a limited number of cycles.

This kit is formulated to amplify the genome of a single cell. WGA from a single cell often results in a million-fold amplification yielding microgram quantities of gDNA. After purification, the Single Cell WGA product can be analyzed in a manner similar to any genomic or chromosomal DNA sample. A number of downstream applications may be performed including TaqMan® assays, microsatellite analysis, SNP analysis, sequencing, comparative genomic hybridization, etc.¹,²

Materials and Reagents Required but not Provided
- Thermal cycler
- Isolated single cells
- Spectrophotometer
- 0.2 mL or 0.5 mL thin-walled PCR tubes or PCR multiwell plate
- Dedicated pipettes
- PCR pipette tips

Precautions and Disclaimer
The Single Cell GenomePlex WGA Kit is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
All components should be stored at −20 °C. When thawed for use, components should be kept on ice. The stability of the Library Preparation Enzyme will be affected if stored warmer than −20 °C or allowed to remain for long periods at temperatures over 4 °C.

Reagents Provided

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Catalog Number</th>
<th>10 Rxn</th>
<th>50 Rxn</th>
<th>500 Rxn</th>
</tr>
</thead>
<tbody>
<tr>
<td>10× Single Cell Lysis &amp; Fragmentation Buffer</td>
<td>L1043</td>
<td>350 μL</td>
<td>1.8 mL</td>
<td>18 mL</td>
</tr>
<tr>
<td>Proteinase K Solution</td>
<td>P4850</td>
<td>25 μL</td>
<td>110 μL</td>
<td>1 mL</td>
</tr>
<tr>
<td>1× Single Cell Library Preparation Buffer</td>
<td>L0918</td>
<td>25 μL</td>
<td>110 μL</td>
<td>1.1 mL</td>
</tr>
<tr>
<td>Library Stabilization Solution</td>
<td>L7292</td>
<td>12 μL</td>
<td>55 μL</td>
<td>550 μL</td>
</tr>
<tr>
<td>Library Preparation Enzyme</td>
<td>E0531</td>
<td>12 μL</td>
<td>55 μL</td>
<td>550 μL</td>
</tr>
<tr>
<td>10× Amplification Master Mix</td>
<td>A5604</td>
<td>85 μL</td>
<td>410 μL</td>
<td>5 x 820 μL</td>
</tr>
<tr>
<td>WGA DNA polymerase</td>
<td>W3891</td>
<td>55 μL</td>
<td>275 μL</td>
<td>2 x 1.375 mL</td>
</tr>
<tr>
<td>Control DNA (5 ng/μL)</td>
<td>D7192</td>
<td>10 μL</td>
<td>10 μL</td>
<td>10 μL</td>
</tr>
<tr>
<td>Water, Molecular Biology Reagent</td>
<td>W4502</td>
<td>1.5 mL</td>
<td>2 x 1.5 mL</td>
<td>2 x 20 mL</td>
</tr>
</tbody>
</table>
Procedure
The single cell WGA process is divided into lysis & fragmentation, OmniPlex library generation, and PCR amplification. These steps should be carried out sequentially without pause, as storage between steps may allow the ends of the library DNA to degrade. Such degradation will affect subsequent steps. The final WGA DNA should be stored at –20 °C and is as stable as any comparably stored genomic DNA sample.

We recommend that all experiments should be performed along with a positive control DNA sample, such as the Control Human Genomic DNA, Catalog Number D7192, included in this kit.

Single Cell Lysis and Fragmentation
1. Isolate a single cell into a PCR-ready vessel using laser capture micro-dissection, cell sorting, or other method. If sorted, the buffer should be of low ionic strength, such as Tris EDTA (TE) buffer, and in the minimal sort volume.
2. Add a sufficient volume of water to the single cell sample for a final volume of 9 µL.
5. Incubate DNA mix at 50 °C for 1 hour, then heat to 99 °C for EXACTLY four minutes. Note that the incubation is very time sensitive. Any deviation may alter results. Cool on ice. Spin down sample prior to proceeding to Library Preparation.

Library Preparation
6. Add 2 µL of 1× Single Cell Library Preparation Buffer to each sample.
7. Add 1 µL of Library Stabilization Solution.
8. Mix thoroughly and place in thermal cycler at 95 °C for 2 minutes.
9. Cool the sample on ice, consolidate the sample by centrifugation, and replace on ice.
10. Add 1 µL of Library Preparation Enzyme, mix thoroughly, and centrifuge briefly.
11. Place sample in a thermal cycler and incubate as follows:
   - 16 °C for 20 minutes
   - 24 °C for 20 minutes
   - 37 °C for 20 minutes
   - 75 °C for 5 minutes
   - 4 °C hold

12. Remove samples from thermal cycler and centrifuge briefly. Samples may be amplified immediately or stored at –20 °C for three days.

Amplification
13. Add the following reagents to the entire 14 µL reaction:
   - 7.5 µL of 10× Amplification Master Mix
   - 48.5 µL of Water, Molecular Biology Reagent
   - 5.0 µL of WGA DNA Polymerase
14. Mix thoroughly, centrifuge briefly, and begin thermocycling. The following profile has been optimized for a PE 9700 or equivalent thermal cycler:
   - Initial Denaturation 95 °C for 3 minutes
   - Perform 25 cycles as follows:
     - Denature 94 °C for 30 seconds
     - Anneal/Extend 65 °C for 5 minutes
     - Hold 4 °C
   - After cycling is complete, maintain the reactions at 4 °C or store at –20 °C until ready for analysis or purification. The stability of WGA DNA is equivalent to genomic DNA stored under the same conditions.

The quality of the WGA DNA can be qualitatively determined by loading 5-10% (4-8 µL) of the final reaction onto a 1.5% agarose gel. The DNA size should range from 100-1000 bp, with the mean size ~400 bp.

Purification of the final product is recommended before being used in subsequent applications. GenomePlex WGA amplified DNA may be purified with the GenElute™ PCR Cleanup Kit, Catalog Number NA1020, or standard purification methods that isolate single and double stranded DNAs. Once purified, the DNA can be quantified by measuring absorbance, assuming that 1 A_260 unit is equivalent to 50 ng/µL DNA. Measurement techniques such as PicoGreen® dye will often underestimate the actual WGA DNA yield, since single stranded DNA might be generated during amplification.
**Product Profile**

All lots are functionally tested by the amplification of a 100 pg sample of standard human genomic DNA that must yield 4 μg of product. The quality and representation of amplification is determined by real-time PCR using primer sets for eight separate loci. Negative (no template) controls must yield no detectable product.

**References**


**Troubleshooting Guide**

<table>
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<tr>
<th>Observation</th>
<th>Cause</th>
<th>Recommended Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield after cycling</td>
<td>Sample contains salt or inhibitors.</td>
<td>The sorting process may carry over inhibitors or salts that disrupt the process.</td>
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<tr>
<td></td>
<td>Poor quality template</td>
<td>The DNA in the single cell was degraded during the isolation process. The single cell was improperly stored. Performing WGA on more than one sample helps rule out quality issues that are random in nature, but will not help if the sorting process damages the sample.</td>
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<tr>
<td></td>
<td>The post reaction purification was inappropriate.</td>
<td>We recommend GenElute PCR Cleanup Kit, Catalog Number NA1020. The method must retain single and double stranded DNA.</td>
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<tr>
<td></td>
<td>A single cell was not captured.</td>
<td>Ensure you have a single cell present in your PCR-ready tube. In addition, make certain to thoroughly vortex after adding in the single cell lysis/fragmentation buffer.</td>
</tr>
<tr>
<td>qPCR shows significant bias in WGA representation for my gene of interest.</td>
<td>The DNA sample is limited or degraded.</td>
<td>See low yield comments.</td>
</tr>
<tr>
<td></td>
<td>Inappropriate controls</td>
<td>Genomic DNA can only be compared to GenomePlex WGA once the control DNA has been sheared. We recommend using several pooled samples that have been subjected to the fragmentation protocol above (steps 1–5), or comparing against DNA subjected to hydroshearing.</td>
</tr>
<tr>
<td>Negative (no template) control affords product.</td>
<td>Reagents have been contaminated by outside source.</td>
<td>One or more reagents have been contaminated with DNA. While this may not affect your results, a clean no template control can be re-achieved only by replacing the affected component.</td>
</tr>
</tbody>
</table>
Frequently Asked Questions

1. **How does GenomePlex work?**
   Genomic DNA is randomly fragmented and the resulting product is manipulated to attach a common sequence at each DNA end. This library of fragments is amplified using twenty-five rounds of PCR.

2. **What if fragmentation (99 °C step) is allowed to proceed for less or more than four minutes?**
   The four-minute fragmentation time was found to give optimal results over a wide variety of DNA samples. Too little or no fragmentation will afford low yields and poor gene representation in the resulting WGA product. A ten-minute fragmentation step will also give poor WGA yields in almost all cases because a significant fraction of the DNA is now too small or degraded to allow efficient library production.

3. **What is the average size of fragmented DNA?**
   The mean size after the fragmentation step is ~0.4 kb.

4. **Will the GenomePlex process afford product with a negative control (no input DNA)?**
   No product will be generated without input of DNA if the procedure is performed correctly.

5. **How should WGA DNA be purified? Is there a preferred way to quantify GenomePlex DNA?**
   We recommend purifying GenomePlex DNA using Sigma’s PCR cleanup kit (Catalog Number NA1020) before it is used in any downstream process. Once purified, the DNA can be quantified by measuring absorbance, assuming that 1 A_{260} unit is equivalent to 50 ng/μl DNA. Measurement techniques such as PicoGreen will often underestimate the actual WGA DNA yield, since single stranded DNA might be generated during amplification.

6. **How can I store GenomePlex DNA? Where can I stop during the GenomePlex process?**
   The WGA process can be divided into single cell lysis/fragmentation, generation of the OmniPlex library, and PCR amplification. Fragmented DNA should be processed immediately, as the ends of this DNA can degrade and will affect subsequent steps. OmniPlex library DNA, generated in the stepped isothermal reactions, can be stored up to three days at −20 °C without any detectable differences to the process. The final WGA DNA should be stored at −20 °C and is as stable as any comparably stored genomic DNA sample.

7. **I have analyzed my sample for gene representation and have observed allelic dropout.**
   Studies have shown that allelic dropout is inherent in single cell amplification. Sigma’s Single Cell WGA Kit minimizes allelic dropout. Our WGA methodology has been tested via quantitative PCR on multiple single cell WGA samples resulting in 30% allelic dropout.

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