

Single Cell WGA Kit

Kit for the amplification of genomic DNA from single cells.

Research Use Only (RUO)

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Single Cell WGA Kit	Ref. No.: 118710 (10 preps)
	Ref. No.: 118750 (50 preps)
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1. Introduction

BIORON's **Single Cell WGA Kit**, developed and produced at BIORON GmbH, is an improved version of the DOP (degenerated oligo PCR) with optimized primer sequences and BIORON's unique SD Polymerase. This enzyme combines the strand displacement capability with heat stability up to 92 °C. Due to these features it is possible to use this enzyme for both amplification steps without additional pipetting or hands on time. The Kit contains everything that is necessary for amplification of human genomic DNA from single cells or low copy templates. Each Kit contains a 2.5x MM (Master Mix without enzyme); 10 U/μl SD Polymerase; Lysis Buffer and PCR grade water.

The amplification is based on a multiple displacement reaction in combination with specially designed degenerated primers optimized for human genomic DNA. The SD Polymerase is able replace former strands during the synthesis of the new strand. In combination with the temperature stability up to 92 °C the two common reaction steps within a whole genome amplification assay does not need additional hands-on time. This minimizes the risk of contamination and handling errors.

2. Content of the Kit

Ref No	118710 10 preps	118750 50 preps	cap color	Storage
2.5x MM	100 μl	2x250 μl	white	-20 °C
10 U/μl SD Polymerase	10 μl	50 μl	blue	-20 °C
PCR grade H ₂ O	1800 μl	1800 μl	transparent	-20 °C
Single Cell Lysis Buffer	30 μl	150 μl	green	2 - 8 °C

Avoid shearing forces during thawing and handling of genomic DNA in the samples.

3. Additional Material Required

- PCR Cycler
- low binding PCR tubes suitable for the used PCR Cycler
- seals or lids suitable for the PCR tubes
- disposable low binding pipette tips with aerosol barrier
- variable-volume single-channel pipettes
- safety laminar box
- safety equipment according to your local requirements
- heating block
- centrifuge

4. Applications for Single Cell WGA Kit

- Single cell DNA analysis
- Next Generation Sequencing (NGS)
- SNP Genotyping
- qPCR- and PCR-based mutation detection
- STR and RFLP analysis
- Array technologies (comparative genomic hybridization)

5. Storage Conditions and Stability

The Kit has to be stored at -20 °C and is stable until the expiry date.

The Lysis Buffer can be stored at +4 °C for short term and at -20 °C for long term storage.

Thaw the 2.5x MM (Master Mix), Lysis Buffer and water just before use. Keep them at 4 °C and the SD Polymerase at -20 °C until needed and transport in a cooling rack.

Please avoid additional freeze and thaw cycles of the 2.5x MM reaction buffer. If a small number of reactions is needed, aliquoting of the 2.5x MM is sufficient.

Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed.

6. Quality Control

The performance of the BIORON **Single Cell WGA Kit** is monitored routinely on a lot-to-lot basis.

7. Protocol for Reaction Setup

Sample requirements:

The Single Cell WGA Kit Lysis Buffer is designed for a rapid lysis of single cells (e.g. epithelial cells) in a single step. There is no further purification necessary. The lysed cell(s) can be used directly for WGA reaction and following analyses. The used amplification method requires intact or at least long fragments of mammalian cells therefore FFPE originated samples are not adequate.

General guidelines for sample characteristics:

- Genomic DNA from 1-100 cells (e.g. 10-1000 pg DNA from human cells)
- Intact or fragmented single- or double-stranded DNA from mammalian sources
- DNA from cell staining may negatively affect the Kit performance
- DNA from formalin fixed samples must be avoided to achieve optimum results

Cell Lysis:

- Use isolated single cells in 2.5 µl 1x PBS buffer
- Add 2.5 µl Single Cell Lysis Buffer
- Incubate at 70° C for 15 min
- Incubate at room temperature for 15 min
- Spin briefly to collect all the liquid at the bottom of the tube
- Use lysed cells for WGA amplification:

WGA amplification

2.5x MM	10 µl
10 U/µl SD Polymerase	1 µl
Lysed cells	5 µl
H ₂ O	fill up to 25 µl
	25 µl reaction volume

Amplification program:

92 °C	2 min	
92 °C	1 min	6x
30 °C	1 min	
ramp rate 0.3 °C/s from 30 °C to 68°C		
68 °C	3 min	
92 °C	30 s	14x
62 °C	30 s	
68 °C	3 min	
68 °C	2 min	

The products of the amplification can be used directly for PCR. We recommend a 1:10 fold dilution but it can be different depending on your personal workflow. Some applications require purification or quantification. The amplified DNA can be purified with spin columns or filter plates. Please note that the sample includes also short DNA fragments which can be lost during purification steps.

8. Warranty and Guarantee of Products

The manufacturer guarantees the performance of its **Single Cell WGA Kit** in the manner described in this IFU. It is up to the purchaser to determine the suitability of **Single Cell WGA Kit** for its particular use. In case a product fails to perform as warranted by any reason, BIORON's sole obligation and the customer's sole remedy is limited to replacement of product free of charge. BIORON excludes all other warranties. We reserve the right to change, alter, or modify our **Single Cell WGA Kit** to enhance its performance and design. The manufacturer's terms and conditions are available upon request.

9. Limitations of Product Use

The use the product is strictly limited to research purposes only. It is not to be applied for any diagnostic, including human, medical or drug purposes.