GenomiPhi™ Single Cell DNA Amplification Kit

Product Booklet

cytiva.com

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Table of Contents

1 Introduction

Product codes

29108107

29108039

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

NOTICE
This kit is sensitive to small amounts of DNA. Wear gloves at all times during the preparation to avoid contamination.

It is the responsibility of the user to verify the use of the GenomiPhi™ Single Cell DNA Amplification Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

Note: *GenomiPhi Single Cell DNA Amplification Kit is optimized for whole genome amplification of DNA from single mammalian cells. Use of apoptotic cells or low quality DNA (such as degraded DNA, or DNA from formalin fixed paraffin embedded samples) can result in increased amplification bias.*

No amplification product is produced in the absence of template DNA for up to 2 hours amplification. However, if amplification reactions are carried out for more than 2 hours, they may produce some artifact DNA synthesis in no-template controls.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage conditions

Store the kit at -70ºC.

The Enzyme Mix must be stored at -70ºC; all other components may be stored at -20°C. Freshly prepared Lysis Buffer after the addition of DTT Solution should be stored at 4ºC. Thaw components on ice and maintain at 0°C to 4°C during handling.

Expiry

This product has been designed to deliver high quality results for up to 12 months from the manufacturing date. Please refer to the expiration date on the product label.

2 Components

GenomiPhi Single Cell DNA Amplification Kit

Reagent	Cap Color	25 Reactions	100 Reactions
GenomiPhi Single Cell Reaction Buffer	Green	1 × 275 µL	1 × 1.1 mL
GenomiPhi Single Cell Enzyme Mix	Yellow	1 × 25 µL	1 × 100 µL
GenomiPhi Single Cell Amplification Mix	Blue	1 × 25 µL	1 × 100 µL
GenomiPhi Single Cell DTT Solution	Red	1 × 100 µL	1 × 100 µL
GenomiPhi Single Cell Lysis Buffer	Black	1 × 100 µL	1 × 200 µL
GenomiPhi Single Cell Neutralization Buffer	White	1 × 100 µL	1 × 200 µL

Reagents and equipment to be supplied by the user

- Liquid-handling supplies - Sterile vials and pipette tips; pipettes, micro centrifuge.
- Water - Use molecular biology grade water free of contaminating DNases or nucleic acid.
- Ice bucket or cold block - for maintaining GenomiPhi Single Cell Kit reagents at 4°C during the experimental setup.
- Perform all amplification reactions in sterile 0.2 mL micro centrifuge tubes or 96-well PCR plates.

- Thermocycler or Real-time qPCR instrument - for incubations at 30°C and 65°C.

3 Product Description

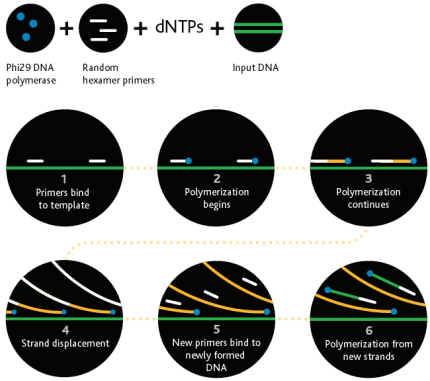


Fig 1. Overview of the GenomiPhi Single Cell DNA Amplification Kit procedure

The basic principle

Fig. 1, on page 6 shows an overview of whole genome amplification by isothermal strand displacement using the GenomiPhi Single Cell DNA Amplification Kit. DNA is briefly denatured and a mastermix containing DNA Polymerase, random modified hexamers, nucleotides and buffers is added to the denatured DNA. Modified random hexamers non-specifically bind to the DNA and isothermal amplification proceeds at 30°C for 2 hours. After amplification the enzyme is heat inactivated during 10 minute incubation at 65°C.

Kit specifications

Typical amplification kinetics with GenomiPhi Single Cell DNA Amplification Kit is shown in *Fig. 2, on page 7*. Microgram quantities of DNA are generated from picogram amounts of starting material in 2 hours. Typical DNA yields from a GenomiPhi Single Cell DNA Amplification Kit reaction are 4–7 µg per 20 µL reaction when starting with a single mammalian cell. Kinetics will vary if crude or un-quantified samples are amplified. Increased reaction times (3 hours) may be helpful for samples such as bacterial cells. Reactions containing no DNA do not produce any product during 2 hours reactions. The average product length is greater than 10 kb. DNA replication is extremely accurate due to the proofreading 3′–5′ exonuclease activity of the DNA Polymerase (3, 4).

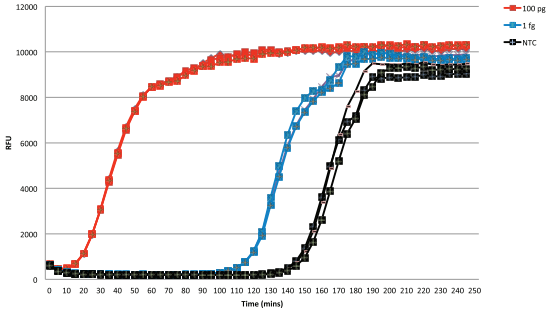


Fig 2. Real-time amplification of 100 pg and 1 fg of human gDNA

Fig. 2, on page 7 shows the comparison of amplification kinetics of 100 pg and 1 fg of human gDNA with a no template control (NTC). GenomiPhi Single Cell DNA Amplification Kit is sensitive up to amplification of 1 fg of gDNA.

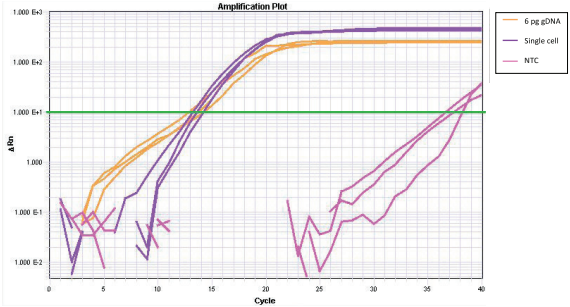
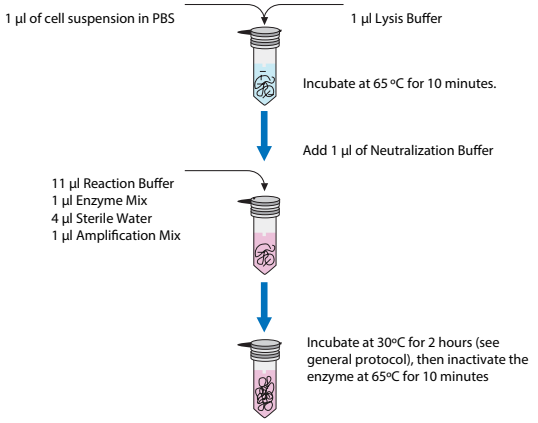


Fig 3. Real-time amplification of a single mammalian cell using GenomiPhi Single Cell DNA Amplification Kit

Fig. 3, on page 8 shows the real-time amplification of single female human cells with a NTC. Amplification from a single cell reach threshold at around 10 cycles (1 cycle = 5 minutes).

4 Protocols

Schematic representation of GenomiPhi Single Cell DNA Amplification Kit protocol.



Whole genome amplification of mammalian cells when isolated using dilution method

The steps outlined below describe a general protocol for amplifying DNA from mammalian cells. This protocol should be considered a starting point for optimizing the reaction in your laboratory.

Preparation of Lysis Buffer

Step	Action
1	Add 1µL of GenomiPhi Single Cell DTT Solution to 9 µL of GenomiPhi Single Cell Lysis Buffer, vortex briefly and spin down. ***Note:*** *Lysis Buffer can be used for 6 weeks when stored at 4°C. Prepare fresh Lysis Buffer after 6 weeks by mixing GenomiPhi Single CellDTT Solution and GenomiPhi Single Cell Lysis Buffer in 1:10 ratio.*
2	Proceed with the next part of the protocol.

Cell lysis step

Step	Action
1	Count cells and dilute with 1 × PBS buffer to required final concentration (1–1000 cells/µL).
2	Transfer 1 µL of cell suspension into 0.2 mL PCR tube and add 1 µL of Lysis Buffer.
3	Briefly centrifuge the tube and incubate at 65°C for 10 minutes and then add 1 µL GenomiPhi Single Cell Neutralization Buffer. ***Note:*** *Exceeding the incubation time or temperature can damage the DNA.*
4	Proceed with the next part of the protocol.

Amplification step

Step	Action
1	Add 1 µL of GenomiPhi Single Cell Enzyme Mix and 4 µL of sterile water to 11 µL of GenomiPhi Single CellReaction Buffer. ***Note:*** *1 µL of 10 × SYBR green I can be optionally added to perform real-time amplification. If SYBR green is added then add only 3 µL of water.*

Optional Clean-Up Step

In order to degrade any potential DN contaminants introduced during the set up, master mix containing GenomiPhi Single Cell Enzyme Mix, Reaction Buffer and water can be incubated at 30°C for 60 minutes prior to addition of Single Cell GenomiPhi Amplification Mix.

Step	Action
1	Add 1 µL of GenomiPhi Single Cell Amplification Mix to 16 µL of master mix containing GenomiPhi Single Cell Enzyme Mix, Reaction Buffer and water and mix well by pipetting the solution up and down several times. ***Note:*** *Do not vortex!*
2	Immediately add all 17 µL of this reaction mix to 3 µL template DNA (from *Cell lysis step, on page 10*).
3	Briefly centrifuge the reaction mixture to remove any air bubbles and incubate at 30ºC for 2 hours.

Step	Action
4	Heat-inactivate the reaction by incubating at 65ºC for 10 minutes. ***Note:*** *For real-time amplification, incubate the tubes in a real-time qPCR instrument.*

Whole genome amplification of mammalian cells when isolated using FACS

Preparation of Lysis Buffer

Step	Action
1	Add 1 µL of GenomiPhi Single Cell DTT Solution to 9 µl of GenomiPhi Single CellLysis Buffer, vortex briefly and spin down. ***Note:*** *Freshly prepared Lysis Buffer can be used for 6 weeks when stored at 4°C. Prepare fresh Lysis Buffer after 6 weeks by mixing GenomiPhi Single Cell DTT Solution and GenomiPhi Single Cell Lysis Buffer in 1:10 ratio.*
2	Prepare 1:1 dilution of Lysis Buffer with sterile water.
3	Proceed with the next part of the protocol.

Cell lysis step

Step	Action
1	Add 2 µL of diluted Lysis Buffer to the appropriate wells of a PCR Plate.

Step	Action
2	Dispense cells directly into the 2 µL of Lysis Buffer.
3	Briefly centrifuge the plate and incubate at 65°C for 10 minutes and then add 1 µL GenomiPhi Single Cell Neutralization Buffer. ***Note:*** *Exceeding the incubation time or temperature can damage the DNA.*
4	Proceed with the next part of the protocol.

Amplification step

Step	Action
1	Refer to *Whole genome amplification of mammalian cells when isolated using dilution method, on page 9*.

GenomiPhi Single Cell DNA Amplification Kit can be used for whole genome amplification of single microbial cells.

Refer to *Whole genome amplification of mammalian cells when isolated using dilution method, on page 9* for the preparation of Lysis Buffer and reaction mix.

Step	Action
1	Bacterial cell lysis can be performed by mixing bacterial cell (1-1000) with Lysis Buffer and immediately freezing at -70°C for 1 hour.

Step	Action
2	After 1 hour thaw the cell lysis solution and immediately incubate at 65°C for 10 minutes.
3	After adding the reaction mix to the cell lysate, incubate the reaction at 30°C for 3.5–4 hours and heat-inactivate the reaction by incubating at 65°C for 10 minutes.

Amplification of DNA (<10 ng)

Preparation of Lysis Buffer

Step	Action
1	Refer to *Whole genome amplification of mammalian cells when isolated using dilution method, on page 9*.
2	Proceed with the next part of the protocol.

DNA denaturation step

Step	Action
1	Dilute DNA to required concentration using sterile water or 1 × Tris- EDTA Buffer (TE) and add 1 µL of diluted DNA to 0.2 mL tube.
2	Add 1 µL of Lysis Buffer and incubate at room temperature for 10 minutes.
3	Add 1 µL GenomiPhi Single Cell Neutralization Buffer.
4	Proceed with the next part of the protocol.

Amplification step

Step	Action
1	Refer to *Whole genome amplification of mammalian cells when isolated using dilution method, on page 9*

Quantification of amplification products

Quantification is generally not required as every reaction will yield approximately the same amount of DNA. Quant-iT PicoGreen® dsDNA quantification reagent (Invitrogen, P7581) is recommended if accurate quantitation is required.

Note: *Quantification of non-purified amplification products by UV absorption will generate inaccurate results due to the presence of unused hexamers in the completed reaction.*

Prepare TE buffer

Step	Action
1	Dilute the concentrated 20 × TE buffer included in the kit to 1× concentration using water. ***Note:*** *Use molecular biology grade DNase free water when preparing the dilution to ensure accurate quantification.*
2	Proceed with the next part of the protocol.

Prepare 1:25 dilution of PicoGreen reagent

Step	Action
1	Determine the required volume of a 1:25 dilution of PicoGreen reagent. • Volume = 100 µL/sample × # of samples
2	Determine the volume of stock PicoGreen reagent necessary to produce the required volume of a 1:25 dilution • Volume = (volume of required dilution)/25
	 **NOTICE** Reagent adsorbs to glass surfaces. Use plastic ware only. Protect the solution from light at all times.
3	Proceed with the next part of the protocol.

Prepare the λ DNA standard curve

Step	Action
1	Dilute the λ DNA standard supplied in the Quant-iT PicoGreen kit to a 10 ng/µL working solution. Use this working stock to prepare a standard curve (see example table).

Step	Action
2	Add 100 µL of each dilution to each well of the assay plate.

Standard Number	λ DNA (ng)	λ DNA (10 ng/µL)	1 × TE bufer
1	600	60 µL	40 µL
2	500	50 µL	50 µL
3	400	40 µL	60 µL
4	200	20 µL	80 µL
5	100	10 µL	90 µL
6	50	5 µL	95 µL
7	25	2.5 µL	97.5 µL
8	0	0µL	100 µL

3	Proceed with the next part of the protocol.

Dilute the GenomiPhi Single Cell amplification products

Step	Action
1	Dilute the GenomiPhi Single Cell amplification products 1:10 by adding 180 µL of 1 × TE buffer to each amplification reaction. Due to the viscosity of the amplification product, mix amplification products thoroughly by vortexing heavily.
2	Proceed with the next part of the protocol.

Add diluted GenomiPhi amplification products to the assay plate

Step	Action
1	Aliquot 90 µL of 1 × TE buffer into each sample well. Add 10 µL of diluted sample for a final volume of 100 µL. ***Note:*** *Because the amplification product is diluted before the assay, the dilution factor must be taken into consideration when calculating total yields.*
2	Proceed with the next part of the protocol.

Add diluted PicoGreen to sample wells

Step	Action
1	Add 100 µL of the 1:25 dilution of PicoGreen to all wells containing standards and samples. Mix contents well by pipetting up and down.

Step	Action
2	Seal the plate with foil and spin in micro plate centrifuge for 1 minute at < 200 × *g* to eliminate bubbles.

NOTICE
Protect plate from light at all times. The plate must be read 5-10 minutes after addition of PicoGreen reagent to ensure accurate quantification

3	Proceed with the next part of the protocol.

Measure the sample fluorescence

Step	Action
1	Place the sample assay plate into a fluorescence micro plate reader. Set the fluorescence reader at the following parameters: • Excitation wavelength: 480 nm • Emission wavelength: 520 nm • Gain: Optimal

Step	Action
	Note: *If it is not possible to set the instrument gain to optimal, find a way to have the instrument read the sample where the highest DNA concentration generates readings that fall within the linear dynamic range of the instrument.*
2	Proceed with the next part of the protocol.

Calculate the concentration of the amplification product

Step	Action
1	Generate a standard curve of fluorescence versus DNA concentration. Determine the concentration of GenomiPhi Single Cell amplified products from the equation of the line derived from the standard curve.

Purification of whole genome amplified DNA

Step	Action
1	Add 30 µL of 20 mM EDTA to 20 µL of amplified DNA. Mix properly by pipetting up and down several times and transfer the 50 µL DNA into a sterile 1.5 mL tube.
2	Add 5 µL of 3M sodium acetate (1/10th volume) and 137 µL (2.5 × volume) of ice-cold 100% ethanol to 50 µL DNA solution.

Step	Action
3	Mix by inverting the tube several times (do not vortex) and directly centrifuge at high speed (~16000 × g) for 20 minutes.
4	Discard the supernatant and add 500 µL of ice-cold 70% ethanol.
5	Mix by inverting the tube several times and centrifuge at high speed for 5 minutes.
6	Discard the supernatant and re-suspend the DNA in TE buffer or water (don't let the pellet completely dry - this will affect the dissolution of DNA).
7	Incubate the DNA at 4°C for 15–20 minutes and gently mix by pipetting the solution up and down several times.
8	Store at -20°C until further use.
	Note: *GenomiPhi Single Cell amplified DNA is not suitable for purification using membrane-based filtration columns.*

5 Troubleshooting

Problem: Reduced yield/no amplification product

Posible cause	Suggestions
Contamination of template DNA	• Excessive contaminants carried over from the starting material can inhibit the DNA Polymerase. Dilute or clean-up the DNA and re-amplify. • Extending the amplification time will help when inhibitory material is causing reduced yields.
Inactive Enzyme	• It is critical that the enzyme be stored properly. The Enzyme Mix should be stored at -70°C. If the material will be consumed within 2 months, -20°C storage may be used. The freezer must not be a frost-free unit. • Perform a control reaction to confirm performance of the enzyme.
Low quality DNA	Amplification kinetics strongly favors intact templates. Avoid template preparation steps that can damage DNA.
Prolonged denaturation	Heating at 65°C for 10 minutes is sufficient to lyse the cells and denature template DNA and facilitate primer annealing. Longer denaturing times can nick the template and decrease the amplification efficiency.

Problem: Poor performance in downstream applications

Posible cause	Suggestions
Degraded/low amounts of template DNA	• In the absence of input DNA or poor quality of input DNA, there will be no or minimal DNA synthesis in the amplification reactions within 2 hours. • Degraded or low amounts of starting DNA template may not amplify consistently or representatively. • Use high quality genomic DNA for amplification.
Inhibition of optimized downstream conditions	• Starting material components can inhibit the amplification reaction. Purify the starting material using a suitable column prior to amplification. • For some downstream applications, components of the GenomiPhi Single Cell reaction will alter previously optimized downstream conditions. Purify the amplification products using a recommended ethanol precipitation method provided in *Purification of whole genome amplified DNA, on page 20*.
Presence of Non-specific amplification product	• Use sterile laboratory equipment and pipette tips. Work in a laminar-flow hood. • Use molecular biology grade sterile water and PBS to prepare all samples. • Perform optional 60 minutes clean-up method (See *Whole genome amplification of mammalian cells when isolated using FACS, on page 12* for details) to remove any potential DNA contaminants introduced during the set up.

6 Related Products

GenomiPhi Products[1]	
GenomiPhi HY Ready-To-Go™ (high yield)	25-6603-24
	25-6603-96
	25-6603-97
GenomiPhi V3 Ready-To-Go	25-6601-24
	25-6601-96
	25-6601-97
GenomiPhi V2 (liquid format)	25660031
GenomiPhi HY (high yield, liquid format)	25660022

DNA Purification Products[1]	
Tissue and cells genomicPrep Mini Spin Kit	28904276
Tissue and cells genomicPrep Midi Flow Kit	28904273
Blood genomicPrep Mini Spin Kit	28904264
TriplePrep	28942544
Bacteria genomicPrep Mini Spin Kit PCR Products[1]	28904258

PCR Products[1]	
PureTaq Ready-To-Go PCR Beads	27955901
Amersham™ Hot Start Mix Ready-To-Go	28900653
GFX™ PCR DNA and Gel Band Purification Kit	28903470
ExoProStar™ - PCR and Sequence Reaction Clean-Up	US78210
ExoProStar 1-Step - PCR and Sequence Reaction Clean-Up	US77702
Taq DNA Polymerase (cloned)	27079804
Amersham Solution dNTPs (multiple formats available)	28406552

[1] please see *cytiva.com* for an overview of available pack sizes.

7 References

1. Dean, F. *et al., Genome Research* **11**, 1095–1099 (2001).
2. Lizardi, P. *et al., Nat. Genet.* **19**, 225–232 (1998).
3. Estaban, J.A. *et al., J. Biol. Chem.* **268**, 2719–2726 (1993).
4. Nelson, J.R. *et al; BioTechniques* **32**, S44-S47 (2002).

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