# illustra™ Single Cell GenomiPhi™ DNA Amplification Kit

## Product booklet

Codes: 29-1081-07
29-1080-39

# Page finder

# 1. Legal



**Patent /licence**

For use only as licensed by Qiagen GmbH. The Phi 29 DNA polymerase enzyme may not be re-sold or used except in conjunction with the other components of the **Single Cell GenomiPhi DNA Amplification Kit.** See U.S. patent Nos. 5,854,033, 6,124,120, 6,143,495, 6,323,009 and related U.S. and foreign patents.


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http://www.gelifesciences.com

GE Healthcare UK Limited.
Amersham Place,
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

## 1.1. Product use restriction/warranty

The **Single Cell GenomiPhi DNA Amplification Kit** components have been designed, developed, and sold **for molecular biology use only.** They are suitable **for *in vitro* use only and not** recommended or intended for diagnosis of disease in humans or animals. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). Do not use internally or externally in humans or animals.

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

# 2. Handling, Safety Warnings and Precautions

## 2.1. Handling, preparation, and storage of starting materials

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

This product and its components should be handled only by persons trained in laboratory techniques and used in accordance with the principles of good laboratory practice. Prepare the reaction in a laminar flow hood to avoid any contamination. Use molecular biology grade clean reagents, sterile reaction tubes and pipette tips. All chemicals should be considered potentially hazardous; therefore, when handling chemical reagents, it is advisable that suitable protective clothing, such as laboratory overalls, safety glasses and gloves be worn. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water. See the appropriate Material Safety Data Sheet for specific recommendations.

**Note: Single Cell GenomiPhi 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.

## 2.2. 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.

## 2.3. 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.

# 3. Components

## 3.1. Kit Contents

**Single Cell GenomiPhi DNA Amplification Kit**

| Cat No. | Cap Color | 25 Reactions | 100 Reactions |
|---|---|---|---|
| Single Cell GenomiPhi Reaction Buffer | Green | 1 × 275 µl | 1 × 1.1 ml |
| Single Cell GenomiPhi Enzyme Mix | Yellow | 1 × 25 µl | 1 × 100 µl |
| Single Cell GenomiPhi Amplification Mix | Blue | 1 × 25 µl | 1 × 100 µl |
| Single Cell GenomiPhi DTT Solution | Red | 1 × 100 µl | 1 × 100 µl |
| Single Cell GenomiPhi Lysis Buffer | Black | 1 × 100 µl | 1 × 200 µl |
| Single Cell GenomiPhi Neutralization Buffer | White | 1 × 100 µl | 1 × 200 µl |

## 3.2. 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 **Single Cell GenomiPhi 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.

# 4. Product Description

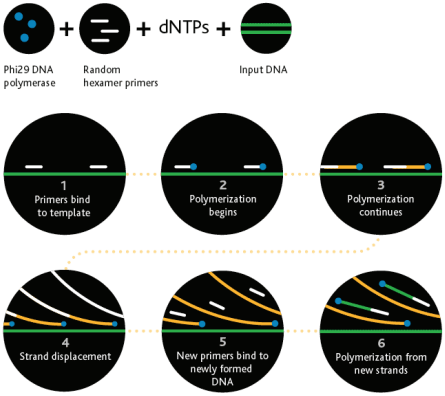


**Figure 1.** Overview of the **Single Cell GenomiPhi DNA Amplification Kit** procedure

## 4.1. The basic principle

Figure 1 shows an overview of whole genome amplification by isothermal strand displacement using the **Single Cell GenomiPhi DNA Amplification Kit**. DNA is briefly denatured and a master-mix 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.

## 4.2. Kit specifications

Typical amplification kinetics with **Single Cell GenomiPhi DNA Amplification Kit** is shown in Figure 2. Microgram quantities of DNA are generated from picogram amounts of starting material in 2 hours. Typical DNA yields from a **Single Cell GenomiPhi 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).

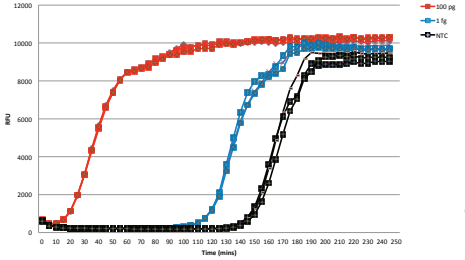


**Figure 2.** Real-time amplification of 100 pg and 1 fg of human gDNA

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

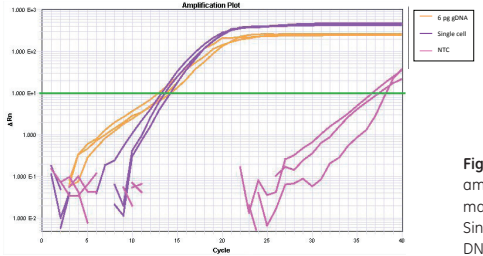


**Figure 3.** Real-time amplification of a single mammalian cell using Single Cell GenomiPhi DNA Amplification Kit

Figure 3 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).

# 5. Protocols

## 5.1. Short Protocol

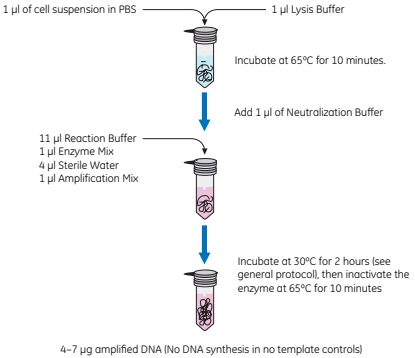


**Figure 4** Schematic representation of **Single Cell GenomiPhi DNA Amplification Kit** protocol.

## 5.2. 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.

### 5.2.1. Preparation of lysis buffer

- Add 1µl of Single Cell GenomiPhi DTT Solution to 9 µl of Single Cell GenomiPhi 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 Single Cell GenomiPhi DTT Solution and Single Cell GenomiPhi Lysis Buffer in 1:10 ratio.

**5.2.2. Cell lysis step**

- Count cells and dilute with 1 × PBS buffer to required final concentration (1–1000 cells/µl).
- Transfer 1 µl of cell suspension into 0.2 ml PCR tube and add 1 µl of lysis buffer.
- Briefly centrifuge the tube and incubate at 65°C for 10 minutes and then add 1 µl Single Cell GenomiPhi Neutralization Buffer.

**Note:** Exceeding the incubation time or temperature can damage the DNA.

**5.2.3. Amplification step**

- Add 1 µl of Single Cell GenomiPhi Enzyme Mix and 4 µl of sterile water to 11 µl of Single Cell GenomiPhi Reaction 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 DNA contaminants introduced during the set up, master mix containing Single Cell GenomiPhi Enzyme Mix, Reaction Buffer and water can be incubated at 30°C for 60 minutes prior to addition of Single Cell GenomiPhi Amplification Mix.

- Add 1 µl of Single Cell GenomiPhi Amplification Mix to 16 µl of master mix containing Single Cell GenomiPhi Enzyme Mix, Reaction Buffer and water and mix well by pipetting the solution up and down several times
  (**Note:** Do not vortex!).
- **Immediately** add all 17 µl of this reaction mix to 3 µl template DNA (from step 5.2.2).
- Briefly centrifuge the reaction mixture to remove any air bubbles and incubate at 30ºC for 2 hours.
- 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.

## 5.3. Whole genome amplification of mammalian cells when isolated using FACS

**5.3.1. Preparation of lysis buffer**

- Add 1 µl of Single Cell GenomiPhi DTT Solution to 9 µl of Single Cell GenomiPhi Lysis 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 Single Cell GenomiPhi DTT Solution and Single Cell GenomiPhi Lysis Buffer in 1:10 ratio.

- Prepare 1:1 dilution of lysis buffer with sterile water.

**5.3.2. Cell lysis step**

- Add 2 µl of diluted lysis buffer to the appropriate wells of a PCR Plate.
- Dispense cells directly into the 2 µl of lysis buffer.
- Briefly centrifuge the plate and incubate at 65°C for 10 minutes and then add 1 µl Single Cell GenomiPhi Neutralization Buffer.

**Note:** Exceeding the incubation time or temperature can damage the DNA.

**5.3.3. Amplification step**

Refer to Section 5.2.3.

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

Refer to Section 5.2 for the preparation of lysis buffer and reaction mix.

Bacterial cell lysis can be performed by mixing bacterial cell (1-1000) with lysis buffer and immediately freezing at -70°C for 1 hour. After 1 hour thaw the cell lysis solution and immediately incubate at 65°C for 10 minutes.

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.

## 5.5. Amplification of DNA (<10 ng)

**5.5.1. Preparation of lysis buffer**

Refer to Section 5.2.1.

**5.5.2. DNA denaturation step**

- 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.
- Add 1 µl of lysis buffer and incubate at room temperature for 10 minutes.
- Add 1 µl Single Cell GenomiPhi Neutralization Buffer.

**5.5.3. Amplification step**

Refer to Section 5.2.3.

## 5.6. 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.

**5.6.1. Prepare TE buffer**

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.

**5.6.2. Prepare 1:25 dilution of PicoGreen reagent**

Determine the required volume of a 1:25 dilution of PicoGreen reagent.

- Volume = 100 µl/sample × # of samples

Determine the volume of stock PicoGreen reagent necessary to produce the required volume of a 1:25 dilution

- Volume = $\frac{\text{volume of required dilution}}{25}$

**Caution:** Reagent adsorbs to glass surfaces. Use plastic ware only. Protect the solution from light at all times.

**5.6.3. Prepare the λ DNA standard curve**

**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).

**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 | 0 µl | 100 µl |

**5.6.4. Dilute the Single Cell GenomiPhi amplification products**
**Dilute** the Single Cell GenomiPhi 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.

**5.6.5. Add diluted GenomiPhi amplification products to the assay plate**
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.

**5.6.6. Add diluted PicoGreen to sample wells**
**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.

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

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

**5.6.7. Measure the sample fluorescence**

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**

**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.

**5.6.8. Calculate the concentration of the amplification product**

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

## 5.7. Purification of whole genome amplified DNA

- 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.
- Add 5 µl of 3M sodium acetate (1/10$^{th}$ volume) and 137 µl (2.5 × volume) of ice-cold 100% ethanol to 50 µl DNA solution.
- Mix by inverting the tube several times (do not vortex) and directly centrifuge at high speed (~16000 × *g*) for 20 minutes.

- Discard the supernatant and add 500 µl of ice-cold 70% ethanol.
- Mix by inverting the tube several times and centrifuge at high speed for 5 minutes.
- 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).
- Incubate the DNA at 4°C for 15–20 minutes and gently mix by pipetting the solution up and down several times.
- Store at -20°C until further use.

**Note:** Single Cell GenomiPhi amplified DNA is not suitable for purification using membrane-based filtration columns.

# 6. Appendix

## 6.1. Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| **Reduced yield/ no amplification product** | **Contamination of template DNA**<br>• Excessive contaminants carried over from the starting material can inhibit the DNA polymerase. Dilute or clean-up the DNA and re-amplify.<br>• Extending the amplification time will help when inhibitory material is causing reduced yields.<br><br>**Inactive Enzyme**<br>• 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.<br>• Perform a control reaction to confirm performance of the enzyme.<br><br>**Low quality DNA**<br>• Amplification kinetics strongly favors intact templates. Avoid template preparation steps that can damage DNA.<br><br>**Prolonged denaturation**<br>• 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 | Possible cause and suggestions |
|---|---|
| **Poor performance in downstream applications** | **Degraded/low amounts of template DNA**<br>• 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.<br>• Degraded or low amounts of starting DNA template may not amplify consistently or representatively.<br>• Use high quality genomic DNA for amplification.<br><br>**Inhibition of optimized downstream conditions**<br>• Starting material components can inhibit the amplification reaction. Purify the starting material using a suitable column prior to amplification.<br>• For some downstream applications, components of the Single Cell GenomiPhi reaction will alter previously optimized downstream conditions. Purify the amplification products using a recommended ethanol precipitation method provided in Section 5.7.<br><br>**Presence of Non-specific amplification product**<br>• Use sterile laboratory equipment and pipette tips. Work in a laminar-flow hood.<br>• Use molecular biology grade sterile water and PBS to prepare all samples.<br>• Perform optional 60 minutes clean-up method (See Section 5.2 for details) to remove any potential DNA contaminants introduced during the set up. |

## 6.2. 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).

## 6.3. Related Products

| **GenomiPhi Products*** | |
|---|---|
| illustra Ready-To-Go™ GenomiPhi HY (high yield) | 25-6603-24<br>25-6603-96<br>25-6603-97 |
| illustra Ready-To-Go GenomiPhi HY (high yield) | 25-6601-24<br>25-6601-96<br>25-6601-97 |
| illustra GenomiPhi V2 (liquid format) | 25-6600-31 |
| illustra GenomiPhi HY (high yield, liquid format) | 25-6600-22 |

| **DNA Purification Products*** | |
|---|---|
| illustra tissue and cells genomicPrep Mini Spin Kit | 28-9042-76 |
| illustra tissue and cells genomicPrep Midi Flow Kit | 28-9042-73 |
| illustra blood genomicPrep Mini Spin Kit | 28-9042-64 |
| illustra triplePrep | 28-9425-44 |
| illustra bacteria genomicPrep Mini Spin Kit PCR Products* | 28-9042-58 |

| PCR Products* | |
|---|---|
| illustra PureTaq™ Ready-To-Go PCR Beads | 27-9559-01 |
| illustra Hot Start Mix™ Ready-To-Go | 28-9006-53 |
| illustra GFX™ PCR DNA and Gel Band Purification Kit | 28-9034-70 |
| illustra ExoProStar™ - PCR and Sequence Reaction Clean-Up | US78210 |
| illustra ExoProStar 1-Step - PCR and Sequence Reaction Clean-Up | US77702 |
| DNA Polymerase (cloned) | 27-0798-04 |
| illustra Solution dNTPs (multiple formats available) | 28-4065-52 |

* please see http://www.gelifesciences.com for an overview of available pack sizes.

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**GE Healthcare offices:**

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala,
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg,
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Japan Coporation
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For your local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

http://www.gelifesciences.com

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