GE Healthcare

illustra GenomiPhi HY DNA Amplification Kit

Product Web Protocol

Codes: 25-6600-20
25-6600-22
25-6600-25

Contents

1. Legal

First published 2006

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1.1. Product use restriction/warranty

The **GenomiPhi HY DNA Amplification Kit** components have been designed, developed, and sold **for research purposes only.** They are suitable **for *in vitro* use only.** 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 **GenomiPhi HY 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

 **This kit is sensitive to small amounts of DNA. Wear gloves and safety glasses 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. 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.

 **GenomiPhi™ HY Amplification Kit is optimized for whole genome amplification from at least 10 ng of high quality genomic DNA template.** Use of less DNA or low quality DNA (such as degraded DNA, or DNA from formalin fixed paraffin embedded samples) can result in amplification bias.

In the absence of template DNA an amplification product will still be produced. This product is the result of amplification of the DNA hexamers and will not interfere with downstream applications. The yield from no DNA reactions will be the same as from with DNA reactions.

 **Wear gloves at all times during the preparation to avoid contamination.**

2.2. Storage conditions

Store the kit at -70ºC.

The enzyme mix must be stored at -70ºC; all other components may be stored at -20ºC. Thaw components on ice and maintain at 0ºC to 4ºC during handling.

2.3. Expiry

This product has been designed to deliver high quality results for up to 18 months from the manufacturing date. Please refer to the detail expiration date on the product label.

3. Components

3.1 Kit Contents

GenomiPhi HY DNA Amplification Kit

	25 Reactions	100 Reactions	1000 Reactions
Cat. No.	**25-6600-22**	**25-6600-20**	**25-6600-25**
Sample Buffer (Green Cap)	**565 µl**	**2.25 ml**	**10 x 2.25 ml**
Reaction Buffer (Blue Cap)	**565 µl**	**2.25 ml**	**10 x 2.25 ml**
Enzyme Mix (Yellow Cap)	**65 µl**	**250 µl**	**10 x 250 µl**
Control DNA (Lambda), 10 ng/µl	**20 µl**	**20 µl**	**10 x 20 µl**

3.2.Reagents to be supplied by the user

Liquid-handling supplies - Vials, pipettes, microcentrifuge, and vacuum centrifuge. Perform all amplification reactions in plastic microcentrifuge tubes (typically 0.5 ml), or in 96-well or 384-well plates suitable for sealing and incubating at 30ºC.

Thermocycler or water baths - for incubations at 30°C, 65ºC and 95ºC.

4. Product description

4.1 The basic principle

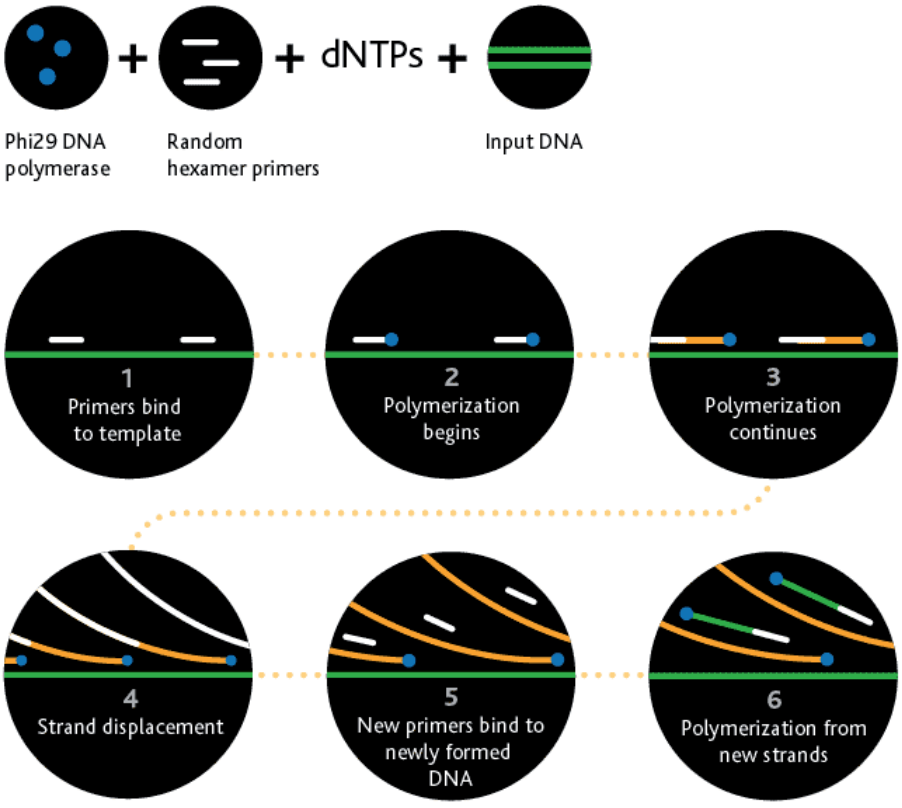

Figure 1 Overview of the **GenomiPhi HY DNA Amplification Kit** procedure

Figure 1 shows an overview of whole genome amplification by isothermal stand displacement using the **GenomiPhi HY DNA Amplification Kit**. DNA is briefly heat-denatured then cooled in sample buffer containing random hexamers that non-specifically bind to the DNA. A master-mix containing DNA polymerase, additional random hexamers, nucleotides, salts and buffers is added and isothermal amplification proceeds at 30°C for 4 hours. After amplification the enzyme is heat inactivated during 10 minute incubation at 65°C.

4.2 Kit specifications

Typical amplification kinetics with **GenomiPhi HY DNA Amplification Kit** is shown in Figure 2 below. Microgram quantities of DNA are generated from nanogram amounts of starting material in only a few hours. Typical DNA yields from a **GenomiPhi HY DNA Amplification Kit** reaction are 45 µg per 50 µl reaction with an average product length of greater than 10 kb. DNA replication is extremely accurate due to the proofreading 3'– 5' exonuclease activity of the DNA polymerase (3, 4). Amplification reactions are generally complete in 3-4 h, yielding approximately 45 µg DNA. Reactions containing no DNA will produce the same amount as with DNA reactions.

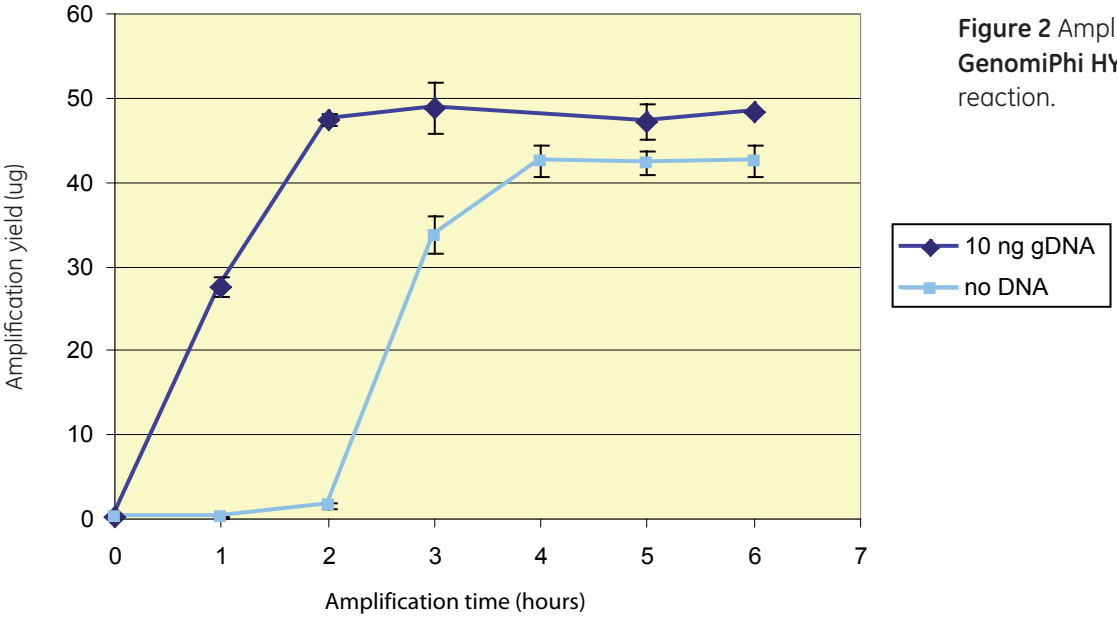

Figure 2 Amplification kinetics of a **GenomiPhi HY DNA Amplification Kit** reaction.

The starting material for **GenomiPhi HY DNA Amplification Kit** reactions can be purified DNA or non-purified cell lysates. Most commercial DNA isolation kits and homemade purification procedures produce suitable DNA for the amplification. Protocols for the amplification of DNA from various clinical samples including blood and buccal cells are available from our web site **(www.genomiphi.com).**

5. Protocols

5.1. Short Protocol

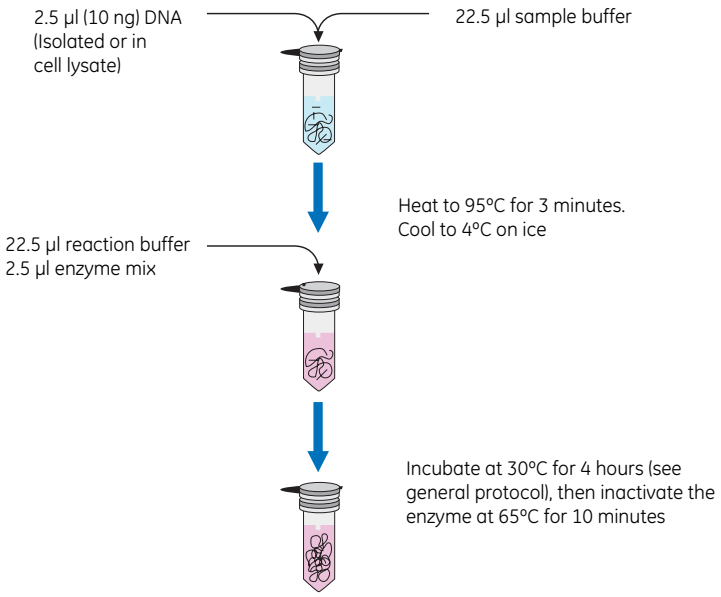

Figure 4 Schematic representation of **GenomiPhi HY DNA Amplification Kit** protocol.

5.2 General Protocol

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

1 Mix sample buffer with template DNA

Add 22.5 µl Sample Buffer to **2.5 µl** of 10 ng template DNA.

22.5 µl Sample Buffer

2.5 µl DNA Template (10 ng)

Template DNA should be resuspended in TE or water.

2 Denature template DNA

Heat the samples to **95°C** for **3 minutes** then cool to **4°C** on ice.

95°C
for 3 minutes

Heating the DNA for longer than 3 minutes or at higher temperatures can cause damage to the DNA.

3 **Prepare the master mix for each amplification reaction**

For each amplification reaction, on ice combine **22.5 µl** of Reaction Buffer with **2.5 µl** of Enzyme Mix.

Prepare the master mix only in sufficient quantities and immediately prior to use. Keep the master mix on ice and discard any unused portion. The master mix contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.

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4 **Transfer master mix to cooled sample.**

Add **25 µl** of prepared master mix from **Step 3** to each cooled sample, on ice.

25 µl
Master Mix

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5 **Incubate for DNA amplification.**

Incubate the samples at **30°C** for **4 hours.**

30°C
for 4 hours

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6 **Phi29 DNA polymerase enzyme.**

Heat the samples to **65°C** for **10 minutes** then cool to **4°C.**

Heating is required to inactivate the exonuclease activity of the DNA polymerase which would otherwise begin to degrade the amplification product.

65°C
for 10 minutes

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7 **Storage of amplified material.**

Store amplification reactions at **-20°C.**

GenomiPhi DNA HY amplification products should be stored and treated as genomic DNA.

Due to the viscosity of the amplification product, dilution in 2 volumes of TE can ease handling. Mix amplification products thoroughly after freezing and thawing.

5.3 Chemical denaturation of template DNA

The steps outlined below describe a general protocol for amplifying template DNA.

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1 **Prepare denaturation and neutralization buffers.**

Denaturation Buffer
400 mM KOH
10 mM EDTA

Neutralization Buffer
200 mM HCl
300 mM Tris-HCl, pH 7.5

2	**Denature template DNA** Mix **2.5 µl** of template (10 ng total) with **2.5 µl** of Denaturation Buffer at **room temperature.** Incubate for 3 minutes.  Mix by pipetting up and down. Do not vortex.	2.5 µl of DNA + 2.5 µl Denaturation Buffer RT for 3 min
3	**Neutralize DNA solution.** Add **5 µl** of Neutralization Buffer.  Mix by pipetting up and down. Do not vortex.	5 µl Neutralization Buffer
4	**Prepare the master mix for each amplification reaction** For each amplification reaction, on ice combine **15 µl** of Sample Buffer and **22.5 µl** of Reaction Buffer, with **2.5 µl** of Enzyme Mix.  Prepare the master mix only in sufficient quantities and immediately prior to use Keep the master mix on ice and discard any unused portion. The master mix contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.	
5	**Transfer master mix to cooled sample.** Add **40 µl** of prepared master mix from **Step 4** to each cooled sample, on ice.	40 µl Master Mix
6	**Incubate for DNA amplification.** Incubate the samples at **30°C** for **4 hours.**	30°C for 4 hours
7	**Phi29 DNA polymerase enzyme.** Heat the samples to **65°C** for **10 minutes** then cool to **4°C**.  Heating is required to inactivate the exonuclease activity of the DNA polymerase which would otherwise begin to degrade the amplification product.	65°C for 10 minutes
8	**Storage of amplified material.** Store amplification reactions at **-20°C.**  GenomiPhi DNA HY amplification products should be stored and treated as genomic DNA. Due to the viscosity of the amplification product, dilution in 2 volumes of TE can ease handling. Mix amplification products thoroughly after freezing and thawing.	

5.4 Quantitation of amplification products

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

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

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1 **Prepare TE buffer.**

Dilute the concentrated TE buffer included in the kit to 1x concentration using water. Dilute 1:20

Use only sterile, distilled, DNase free water when preparing the dilution to ensure accurate quantitation.

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

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

- Volume = 100 µl/sample x # of samples

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

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

Reagent adsorbs to glass surfaces. Use plasticware only. Protect the solution from light at all times.

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

Dilute the λ DNA standard supplied in the kit to a **10 ng/µl** working solution. Use this working stock to prepare a standard curve (see below). **Add 100 µl** of each dilution to each well of the assay plate.

Standard Number	λ DNA (10 ng)	λ DNA (10 ng/µl)	1 x TE
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

4 **Dilute the GenomiPhi HY amplification products twice.**

Dilute the GenomiPhi HY amplification products 1:4 by **adding 150 µl** of 1x TE to each amplification reaction.

Dilute the 1:4 dilution in a separate tube by adding **10 µl** of 1:4 dilution to **90 µl** of 1 x TE.

Serially Dilute
1:4
+
1:10

Due to the viscosity of the amplification product, mix amplification products thoroughly by vortexing heavily.

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

Aliquot 90 µl of 1x TE into each sample well. Add 10 µl of diluted sample for a final volume of 100 µl.

90 µl of TE
+
10 µl of diluted sample

Because the amplification product is diluted **before** the assay, the dilution factor must be taken into consideration when calculating total yields.

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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 microplate centrifuge for 1 minute at < 200 x *g* to eliminate bubbles.

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

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7 **Measure the sample fluorescence.**

Place the sample assay plate into a fluorescence microplate reader.

Set the fluorescence reader at the following parameters:

- Excitation wavelength: **480 nm**
- Emission wavelength: **520 nm**
- Gain: **Optimal**

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.

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8 Calculate the concentration of the amplification product.

Correct the data by subtracting the value obtained for the blank from that of each of the samples. Generate a standard curve of fluorescence versus DNA concentration using the corrected data. Determine the concentration of GenomiPhi HY Kit amplified products from the equation of the line derived from the standard curve.

6. Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Reduced yield/ no amplification product	**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-am plify. • 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 **95°C** for 3 minutes is sufficient to denature template DNA and facilitate primer annealing. Longer denaturing times can nick the template and decrease the amplification efficiency.
Poor performance in downstream applications	**Degraded/low amounts of template DNA** • In the absence of input DNA, there will most likely still be an amplification product. This amplification product will be nonspecific and may not generate expected results in downstream applications. • Degraded or low amounts of starting DNA template may not amplify consistently or representatively. Increase the amount of starting DNA. • Use high quality genomic DNA for amplification. **Inhibition of optimized downstream conditions** • Starting material components can inhibit amplification reaction. Purify the starting material using a suitable column prior to amplification. • For some downstream applications, components of the GenomiPhi HY reaction will alter previously optimized downstream conditions. Purify the amplification products using a suitable column after amplification.

6.2 References

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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

DNA Purification Products

GFX™ Genomic Blood DNA Purification Kit

Nucleon™ BACC Genomic DNA Extraction Kits

GFX™ Micro Plasmid Prep Kit

MicroSpin™ Columns

GFX™ PCR DNA and Gel Band Purification Kit

CyScribe™ GFX™ Purification Kit

ProbeQuant™ G-50 Micro Columns

PCR Products

PureTaq™ Ready-To-Go™ PCR Beads

FideliTaq™ PCR Master Mix

dNTP Set, 100 mM Solution (dATP, dGTP, dCTP, dTTP)

Exo-SAP IT

Taq Polymerase

Sequencing Products

MegaBACE™ DNA Analysis System

DYEnamic™ ET Dye Terminator Cycle Sequencing Kit

Autoseq™ G-50

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25-6600-20WP Rev C 2007