cytiva

Amersham GenomiPhi HY DNA Amplification Kit

Product Booklet

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1 Introduction

Product code

25-6600-20

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.

Safety

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

Storage

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.

Expiry

Kit components are stable for 12 months from date of manufacture when stored at -70ºC.

2 Components

Main components

GenomiPhi™ HY DNA Amplification Kit contains sufficient reagents to perform 100 amplification reactions.

Components

- Sample buffer, 2.25 mL
- Reaction buffer, 2.25 mL
- Enzyme mix , 250 µL
- Control DNA (Lambda, 10 ng/µL), 20 µL

Other materials required

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.

Incubator - For incubations at 30°C.

3 Background

GenomiPhi HY DNA Amplification Kit contains all the components necessary for whole genome amplification by isothermal stand displacement amplification (1,2). Microgram quantities of DNA are generated from nanogram amounts of starting material in only a few hours. Typical DNA yields from a GenomiPhi HY reaction are 40–50 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).

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

Overview

DNA is briefly heat-denatured then cooled in sample buffer containing random hexamers that non-specifically bind to the DNA (See the figure below). 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 (*Fig. 2, on page 6*). After amplification the enzyme is heat inactivated during a 10 minute incubation at 65°C.

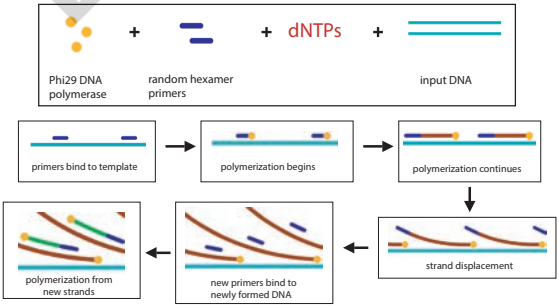

Fig 1. Schematic of GenomiPhi DNA Amplification process

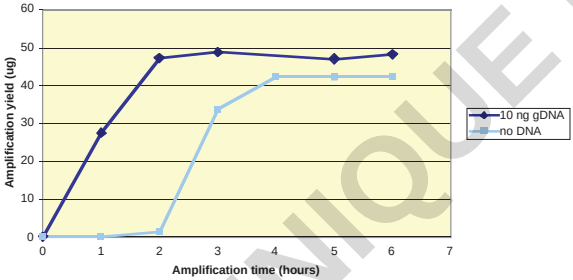

Fig 2. Typical amplification kinetics with GenomiPhi HY Kit. Amplification reactions are generally complete in 4 h, yielding approximately 40–50 µg DNA. Reactions containing no DNA will produce the same amount as with DNA reactions after 4 h amplification, but the product is non-specific, background DNA

4 Protocol

Important Notes. Please read before starting.

- **This protocol 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 down stream applications. The yield from no DNA reactions will be the same as from with DNA reactions.

Performing DNA amplification with GenomiPhi HY DNA Amplification Kit

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.

Step	Action
1	**Mix 2.5 µL of template DNA (10 ng) with 22.5 µL of sample buffer.** ***Note:*** *Template DNA should be resuspended in TE or water.*
2	**Heat the samples to 95ºC for 3 minutes then cool to 4ºC on ice.** ***Note:*** *Heating the DNA for longer than 3 minutes or at higher temperatures can cause damage to the DNA.*
3	**For each amplification reaction, on ice combine 22.5 µL of reaction buffer with 2.5 µL of enzyme mix then add to the cooled sample.** ***Note:*** *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.*

Step	Action
4	**Incubate the samples at 30ºC for 4 hours.** ***Note:*** *Amplification is generally complete in 4 h.*
5	**Heat the samples to 65ºC for 10 minutes then cool to 4ºC.** ***Note:*** *Heating is required to inactivate the exonuclease activity of the DNA polymerase which would otherwise begin to degrade the amplification product.*
6	**Store amplified material at –20ºC.** ***Note:*** *GenomiPhi DNA 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 of the amplification product. Mix amplification products thoroughly after freezing.* ***Note:*** *Purification of the amplification product is generally not required before use in downstream applications. A spin column such as MicoSpin™ G-50 columns (Cytiva, 27-5330-01) may be used to purify the amplification product in cases where reaction components such as the DNA polymerase interfere with downstream application.*

5 Appendices

Quantification of amplification products

Quantification is generally not required as every reaction will yield approximately the same amount of DNA. 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.

Troubleshooting guide

Problems	Possible causes/Solutions
Reduced yield or no amplification product	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 can also help when inhibitory material is causing reduced yields.
Poor performance in down stream applications	• Degraded or low amounts of starting DNA template may not amplify consistently or representatively. Increase the amount of starting DNA and use intact DNA. • Reaction components can inhibit some downstream applications. Purify the amplification products using a suitable column prior to amplification.

6 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)

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