GE Healthcare

illustra™ Ready-To-Go™ GenomiPhi™ HY DNA Amplification Kit

Product booklet

Codes: 25-6603-24
25-6603-96
25-6603-97

Page finder

1. Legal

http://www.gelifesciences.com

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1.1. Product use restriction/warranty.

The **Ready-To-Go 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 **Ready-To-Go 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

Warning: *For research use only.*

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Ready-To-Go GenomiPhi HY DNA 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 a product will still be produced due to amplification of unoccupied hexamers in the reaction. The kinetics of this non-specific amplification are slow and in the presence of

template, non-specific product will be minimal with the reaction massively favouring the desired template.

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

2.2. Storage Conditions

Store the kit at ambient temperature.

Once opened, completely reseal the pouch containing the Ready-To-Go GenomiPhi HY DNA amplification cakes. To ensure maximum shelf life and optimal performance, storage in a desiccator is recommended.

Open product may be stored by resealing the pouch and folding the sealed edge over several times and sealing with a clip.

Store reconstituted Control DNA (Lambda) at -20°C.

For longer-term stability, once opened, store 2× denaturation buffer at 2–8°C.

2.3. Expiry

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

3. Components

3.1. Kit Contents

Ready-To-Go GenomiPhi HY DNA Amplification Kit

Cat. No.	24 Reactions 25-6603-24	96 Reactions 25-6603-96	5 × 96 Reactions 25-6603-97
2× Denaturation buffer	1 × 2.6 ml	1 × 2.6 ml	5 × 2.6 ml
Ready-To-Go GenomiPhi HY cakes	3 × 8-well strips	1 × 96-well plate	5 × 96-well plates
Control DNA (Lambda), 10ng/ul when reconstituted with 50 µl of sterile water	1 × vial	1 × vial	1 × vial
Strips of 8 domed caps	12 strips	12 strips	5 × 12 strips

3.2. Reagents to be supplied by the user

- **Liquid-handling supplies** – Sterile vials and pipette tips; pipettes, micro centrifuge
- **Water** – Use PCR-grade water that is free of contaminating DNases or nucleic acid.
- **Ice bucket or cold block** - for maintaining **Ready-To-Go GenomiPhi HY DNA Amplification Kit** cakes at 4°C during reconstitution and prior to DNA amplification.
- Amplification reactions should be performed in the wells provided. Individual strips can be split from the rest of the plate by bending the strip upwards, **carefully** cutting the seal between adjacent columns of wells, then snapping-off the required strip of 8 wells

For fewer reactions, carefully tap out cake into suitable PCR tube, taking precautions to avoid introducing contaminating DNA (as section 2.1).

- **Thermocycler or water baths** - for incubations at 30°C, 65°C and 95°C.
- **TE-^{1} buffer** - 10 mM Tris-HCl pH 8.0, 0.1 mM EDTA.

4. Product description

4.1. The Basic Principle

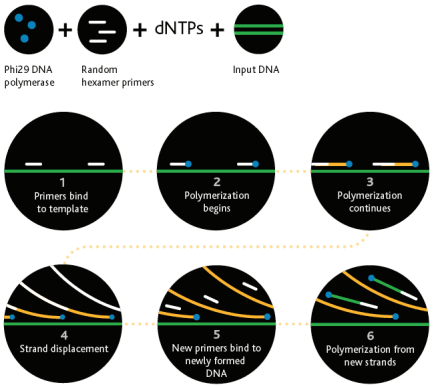

Figure 1. Overview of the **Ready-To-Go GenomiPhi HY DNA Amplification Kit** procedure.

Figure 1 shows an overview of whole genome amplification by isothermal stand displacement using the **Ready-To-Go GenomiPhi HY DNA Amplification Kit**. DNA is briefly heat-denatured in denaturation buffer then cooled. This is added to the freeze-dried cake which contains DNA polymerase, random hexamers, nucleotides, salts and buffers. Isothermal amplification proceeds at 30°C for 4 hours. After amplification the enzyme is heat inactivated during a 10 minute incubation at 65°C.

4.2. Kit specifications

Typical amplification kinetics with **Ready-To-Go GenomiPhi HY DNA Amplification Kit** is shown in Figure 2. Microgram quantities of DNA are generated from nanogram amounts of starting material in only a few hours. Typical DNA yields from a **Ready-To-Go GenomiPhi HY**

DNA Amplification Kit reaction are 40–60 µg per 50 µl reaction with an average product length of greater than 10kb. 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 hours.

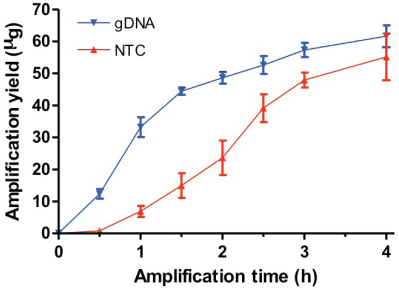

Figure 2. Amplification kinetics of a **Ready-To-Go GenomiPhi HY DNA Amplification Kit** reaction.

The starting material for **Ready-To-Go 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.

5. Protocols

5.1. Short Protocol

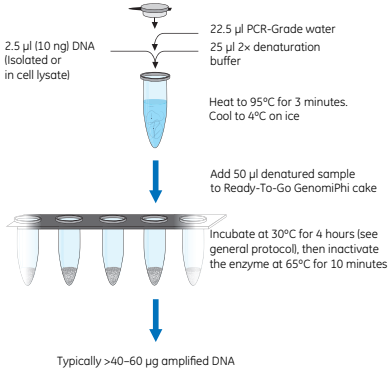

Figure 3. Schematic representation of **Ready-To-Go GenomiPhi HY DNA Amplification Kit** protocol.

5.2. General Protocol

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

This protocol should be considered a starting point for optimizing the reaction in your laboratory.

DNA should be resuspended in PCR-grade water or TE-^{1} buffer. The Denaturation buffer is supplied as a 2× solution to allow some flexibility in volume of DNA that can be used.

Note: The total volume of template DNA and water should equal 25 µl.

When required, the Control DNA should be reconstituted with 50 µl PCR-grade water. A screw-cap has been provided to re-seal the tube. Store at -20°C after reconstitution (in aliquots if so desired).

1. Mix 2× denaturation buffer with template DNA.

Add 25 µl 2× Denaturation Buffer to **10 ng** template DNA.

25 µl denaturation buffer

Add PCR-grade water **to a total volume of 50 µl water.**

22.5 µl PCR-grade water

Template DNA should be resuspended in water or TE-[1] buffer. If sample DNA concentration is less than 10 ng/µl more volume can be used, the total volume of DNA plus water should equal 50 µl.

2.5 µl DNA template (10 ng)

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. Reconstitute the Ready-To-Go GenomiPhi HY cake with the denatured template DNA.

Add the **50 µl** of cooled denatured DNA template from **Step 2** to each cake. Seal wells with domed caps provided. Keep each amplification reaction on ice prior to incubation at 30°C.

50 µl denatured DNA

The reconstituted **Ready-To-Go GenomiPhi HY cake** contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.

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

30°C for 4 hours

5. Inactivate the Phi29 DNA polymerase enzyme.
Heat the samples to **65°C** for **10 minutes** then cool to **4°C**.

65°C for 10 minutes

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

6. Storage of amplified material
Store amplification reactions at **-20°C**.

Ready-To-Go 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 buffer can ease handling. Mix amplification products thoroughly after freezing and thawing. Minimize freeze-thaw cycles.

5.3. Chemical Denaturation and Amplification of Template DNA

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

1. Prepare chemical denaturation solution, neutralization buffer and potassium chloride.

Chemical Denaturation Solution
400 mM KOH
10 mM EDTA

Neutralization Buffer
400 mM HCl
600 mM Tris-HCl, pH 7.5
(prepared by mixing 4 ml of 1 M HCl and 6 ml of 1 M Tris-HCl pH 7.5)

Potassium Chloride
150 mM KCl

2. Denature template DNA.

Mix **2.5 µl** of template (10 ng) with **2.5 µl** of Chemical Denaturation Solution.

2.5 µl of DNA +
2.5 µl Denaturation solution

Incubate at room temperature for 3 minutes

RT for 3 minutes


Mix by pipetting up and down. Do not vortex.

3. Neutralize DNA solution.

Add **5 µl** of Neutralization Buffer and store on ice.

5 µl of Neutralization buffer


Mix by pipetting up and down. Do not vortex.

4. Add PCR-grade water and potassium chloride

Add **15 µl** of PCR-grade water and **25 µl** 150 mM KCl.

15 µl of PCR-grade water +
25 µl of potassium chloride

Keep on ice.

5. Reconstitute the Ready-To-Go GenomiPhi HY cake with the denatured template DNA.

Add the **50 µl** of denatured DNA template from **Step 4** to each cake. Seal wells with

50 µl denatured DNA

domed caps provided. Keep each amplification reaction on ice prior to incubation at 30°C.


The reconstituted **Ready-To-Go GenomiPhi HY cake** contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.

6. Incubate for DNA amplification.
Incubate the samples at 30ºC for **4 hours.**

30°C for 4 hours

7. Inactivate Phi29 DNA polymerase enzyme.
Heat the samples to **65ºC** for **10 minutes** then cool to **4ºC**.

65°C for 10 minutes


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

8. Storage of the amplified material.
Store amplification reactions at **-20°C**.


Ready-To-Go 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. Minimize freeze-thaw cycles.

5.4. DNA Amplification from Blood Cells

Note: Components of blood (e.g. heme) can be inhibitory to the GenomiPhi reaction.

Amplification is faster and more reproducible when the blood sample is diluted before amplification.

A reaction time of 4 hours is suggested.

1. Prepare cell lysis solution, neutralization buffer and potassium chloride solution.

Cell lysis solution
400 mM KOH
10 mM EDTA
100 mM DTT

Neutralization Buffer
400 mM HCl
600 mM Tris-HCl, pH 7.5
(prepared by mixing 4 ml of 1 M HCl and 6 ml of 1 M Tris-HCl pH 7.5)

Potassium Chloride
150 mM KCl

2. Cell lysis

Dilute blood 3 times with physiological buffer, e.g., PBS (137 mM NaCl; 2.7 mM KCl; 10 mM phosphate buffer pH 7.4).

Dilute blood × 3

Add one part of this mixture with one part of cell lysis solution.

1 µl of diluted blood cells +
1 µl cell lysis solution.

Mix well with gentle tapping and incubate on ice for 10 minutes

10 minutes on ice


Mix by pipetting up and down. Do not vortex.

3. Neutralize cell lysate.

Add one part of neutralization buffer to the cell lysate, mix well and store on ice.

1 µl of Neutralization buffer


Mix by pipetting up and down. Do not vortex.

4. Add PCR-grade water and potassium chloride.

To **3 µl** from **Step 3**, add **22 µl** of PCR-grade water and **25 µl** 150 mM KCl.
Keep on ice.

22 µl of PCR-grade water +
25 µl of potassium chloride

5. Reconstitute the Ready-To-Go GenomiPhi HY cake with the denatured template DNA.

Add the **50 µl** of denatured DNA template from **Step 4** to each cake. Keep each amplification reaction on ice prior to incubation at 30°C.

50 µl denatured DNA

The reconstituted **Ready-To-Go GenomiPhi HY cake** contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.

6. Incubate for DNA amplification.

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

30°C for 4 hours

7. Inactivate the Phi29 DNA polymerase enzyme.

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

65°C for 10 minutes


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

8. Storage of the amplified material.
Store amplification reactions at **-20°C**.

Ready-To-Go GenomiPhi DNA HY amplification products should be stored and treated as genomic DNA. Minimize freeze-thaw cycles.

5.5. DNA Amplification from Blood-Blotted Paper (e.g. Whatman's FTA Paper or Guthrie card)

1. Prepare cell lysis solution, neutralization buffer, 2× denaturation buffer and TE-^{1} buffer.

Cell lysis / denaturation solution
400 mM KOH
10 mM EDTA
100 mM DTT

Neutralization Buffer
400 mM HCl
600 mM Tris-HCl, pH 7.5

2× Denaturation buffer
20 mM Hepes, pH8.25
1.0 mM ETDA
0.02% Tween-20
150 mM KCl

TE-^{1} buffer
10 mM Tris-HCl pH 8.0
0.1 mM EDTA

2. Punch processing.

From the blood-blotted and dried FTA paper, punch out 1.2 or 3 mm diameter disks.

Wash the disks three times for 5 minutes with FTA purification reagent (GE catalog number WB120204) in a micro centrifuge tube (using 200 µl for 1.2 mm, and 500 µl for 3 mm disks).

Wash each disk twice for 5 minutes with TE-^{1} buffer. Remove as much fluid as possible at the end of the second TE-^{1} buffer wash.


Mix by pipetting up and down. Do not vortex.

3. Denaturation.

Add 20 µl cell lysis /denaturation solution to the damp disks from Step 2.

20 µl cell lysis solution

Mix well with gentle tapping and incubate on ice for 10 minutes.


Mix by pipetting up and down. Do not vortex.

4. Neutralization.

Add 20 µl neutralization buffer to the cell lysate.

20 µl Neutralization Buffer +
20 µl PCR-grade water

Add 20 µl PCR-grade water, mix well and store on ice.


Mix by pipetting up and down. Do not vortex.

5. Add 2× denaturation buffer.

To 5–10 µl from Step 4, add PCR-grade water to give 25 µl volume. Add 25 µl 2× denaturation buffer.

Keep on ice.

6. Reconstitute the Ready-To-Go GenomiPhi HY cake with the denatured cell lysate DNA.

Add the **50 µl** of denatured DNA template from **Step 5** to each cake. Keep each amplification reaction on ice prior to incubation at 30°C.

50 µl denatured DNA

The reconstituted **Ready-To-Go GenomiPhi HY cake** contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.

7. Incubate for DNA amplification.

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

30°C for 4 hours

8. Inactivate the Phi29 DNA polymerase enzyme.

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

65°C for 10 minutes

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

9. Storage of the amplified material.

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

Ready-To-Go GenomiPhi DNA HY amplification products should be stored and treated as genomic DNA. Minimize freeze-thaw cycles.

5.6. Quantification of Amplification Products

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

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

1. Prepare TE buffer.

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

Prepare 1x TE buffer

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

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

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

3. Prepare the λ DNA standard curve.

Dilute the λ DNA standard supplied in the Quant-iT PicoGreen® kit to a **10 ng/µ**l 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 (ng)	λ DNA (10 ng/µl)	1 × 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 Ready-To-Go GenomiPhi HY amplification products twice.

Dilute the Ready-To-Go GenomiPhi HY amplification products 1:4 by **adding 150 µl** of 1 × TE to each amplification reaction.

Serially dilute 1:4 + 1:10

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

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

5. Add diluted Ready-To-Go GenomiPhi amplification products to the assay plate.

Aliquot **90 µl** of 1 × TE into each sample well. **Add 10 µl** of diluted sample for a final volume of **100 µ**l.

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.

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 to eliminate bubbles.

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

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.

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 Ready-To-Go GenomiPhi HY Kit amplified products from the equation of the line derived from the standard curve.

6. Appendix

6.1. Troubleshooting

Problems	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-amplify. • Extending the amplification time will help when inhibitory material is causing reduced yields. **Inactive Enzyme** • It is critical that the **Ready-To-Go GenomiPhi HY DNA Amplification Kit** freeze-dried cakes are stored properly. Once opened, completely reseal the pouch, fold the sealed edge over several times and seal with a clip. In high humidity environments, store unopened and resealed pouches in a desiccator to maximise product life. • Each cake should be a bright white solid having the shape of the base of the well. Cakes that have been exposed to moisture will become increasingly more translucent in appearance and this will impact both stability and performance. • Perform a control (Lambda DNA) reaction to confirm performance of the **Ready-To-Go GenomiPhi HY DNA Amplification Kit**.

Problems	Possible cause and suggestions
Reduced yield/ no amplification product. *Continued*	**Low quality DNA** • Amplification kinetics strongly favor 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. Longer denaturation 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 non-specific 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** • Components of the Ready-To-Go GenomiPhi HY reaction may affect previously optimized conditions for some downstream applications. Purify the amplification products after amplification.

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 V3	25-6601-24 25-6601-96 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 blood genomicPrep Midi Flow Kit	28-9042-61
illustra triplePrep Kit	28-9425-44
illustra bacteria genomicPrep Mini Spin Kit	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
Taq 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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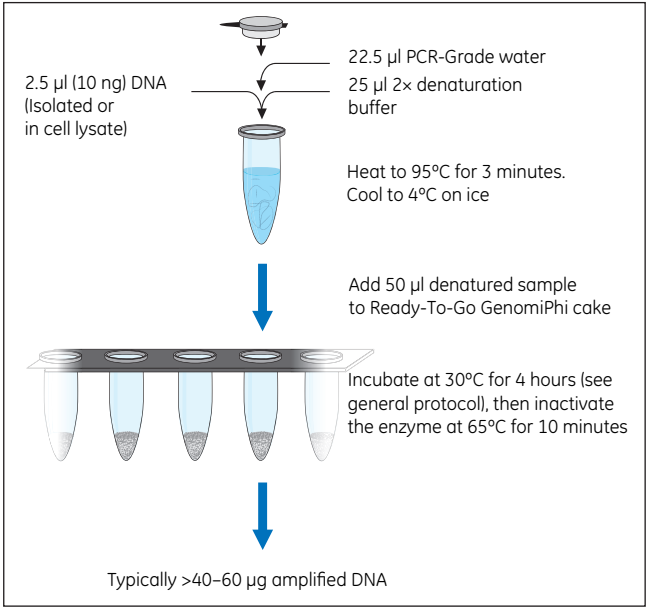

1.1. Product use restriction/warranty.

The **Ready-To-Go GenomiPhi HY DNA Amplification Kit** components have been designed, developed, and sold **for research purposes only**. They are suitable 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 **Ready-To-Go 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.

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