Ver. 1903-00

HelixAmp™ Genome Amplifier (WGA)

Kit Contents

HelixAmp™ Genome Amplifiler (WGA)		
Cat. No.	**HGA001-50 (50 rxns)**	**HGA001-100 (100 rxns)**
8x DB [WGA]	50 µl	100 µl
10x NB [WGA]	100 µl	200 µl
Enzyme Mix [WGA]	50 µl	100 µl
Reaction Buffer [WGA]	0.6 ml	1.2 ml
Primer Mix [WGA]	50 µl	100 µl
dNTP Mix (each 10 mM)	100 µl	200 µl
Control DNA [WGA]	20 µl	40 µl
Instruction for Use	1ea	1ea

Description

HelixAmp™ Genome Amplifier is suitable to amplify the whole genome from very little amount of genomic DNA. The genome amplification is performed by *Phi29* DNA polymerase with an isothermal MDA (multiple displacement amplification)–based mode. Because of the strand displacement activity, strong 3′→5′ exonuclease activity and extreme processivity, *Phi29* DNA polymerase could produce DNA up to 100 kb long with high fidelity. About 1,000 fold amplified DNA can be obtained from 10 ng genomic DNA within 90 min using this kit. The amplified genome can be directly applied to downstream genetic analysis works including PCR, genotyping, and library constructions.

- Fast and uniform amplification across entire genome
- Multiple Displacement Amplification by *Phi29* DNA polymerase
- Whole Genome Amplification from small amounts of sample

Application

Genotype analysis
PCR and Real-time PCR
Construction of genomic library

Store

-20℃

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Quality Control Assay

Functional assay

HelixAmp™ Genome Amplifier was tested for the amplification of whole genome using 10 ng human genomic DNA at 30℃ for 1.5 hours.

Evaluation of amplified DNA

- **Agarose gel electrophoresis** : The integrity of the amplified DNA is assessed by agarose gel electrophoresis.
- **Quantification of amplified DNA** : The DNA amplified with HelixAmp™ Genome Amplifier are quantified using a quantitative Real-time PCR Assay.

Quality authorized by Yountaek Go 

Protocol

1. Preparation of sample and reagents

1) Prepare the 1x DB and 1x NB by diluting the 8x DB and 10x NB with RNase-free Water.

[Example]

1x DB (for up to 40 rxns)	
8x DB	5 µl
RNase-free water	35 µl
Total volume	40 µl

1x NB (for up to 40 rxns)	
10x NB	8 µl
RNase-free water	72 µl
Total volume	80 µl

※ Caution : Do not use the 1x DB and 1x NB older than 1 week.

2) Prepare 1 µl of template DNA (10 ~ 50 ng/µl) in a microcentrifuge tube.

Recommended amount of template DNA

Human genomic DNA : >10 ng

Plant genomic DNA : >10 ng

Bacterial genomic DNA : >20 ng

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<NOTE>

☞ This protocol is optimized for whole genome amplification from >10 ng genomic DNA template. Recommend to validate the concentration of template DNA comparing with the control DNA provided in this kit by agarose gel electrophoresis rather than spectrophotometry. The DNA concentration determined by spectrophotometry should be different according to the purity and integrity of DNA. See the Troubleshooting Guide for the preparation of genomic DNA.

☞ Control reaction can be set up using control genomic DNA (50 ng/μl) provided in this kit.

2. Preparation of reaction Mixture

1) **Add 1 μl of 1x DB** to the DNA sample and Mix. Briefly vortex and spin down.
2) Incubate at room temperature for 3 min.
3) **Add 2 μl of 1x NB** to the sample and Mix. Briefly vortex and spin down.
4) **Add 12 μl of Reaction Buffer** and **2 μl of dNTP Mix** (each 10 mM).
5) **Add 1 μl of Primer Mix and 1 μl of Enzyme Mix**. Briefly vortex and spin down.
6) Incubate at 30℃ for 1.5 ~ 2 hours.
   ※ Recommend to incubate at an incubator or PCR machine. Do not incubate at a water-bath.
7) Inactivate the enzyme at 65℃ for 3 min.
   ※ For PCR, dilute the amplified DNA 10 ~ 25 fold with TE (10 mM Tris/1 mM EDTA, pH8.0) or D.W. Use 1 ~ 3 μl of the diluted DNA for PCR template.
8) Store amplified DNA at -20℃.

Troubleshooting Guide

ISSUE 1. Little or no high-molecular weight WGA product.

Possible cause	For example	Comments and Suggestions
Possible inhibitor in the genomic DNA sample	Contaminants introduced by the purification procedure of genomic DNA : Salt, phenol, SDS, EDTA > 1 mM : In plants, metabolite co-purified with genomic DNA.	Clean-up the genomic DNA. In plants, recommend to use the fresh, healthy, and young tissues.
Low-quality and/or fragmented template DNA	If the low-quality and/or fragmented template DNA, such as DNA purified from the old sample or the formalin-fixed, paraffin-embeded samples, are used in WGA reaction, it may be caused the reduced or no high-molecular weight WGA product.	Use the high-quality of genomic DNA or intact genomic DNA. Increase the amount of template DNA.
Inactivation of Enzyme	Enzyme Mix is stable at -20℃ and must be kept on ice during the preparation of reaction Mixture. After use, Enzyme Mix must be stored at -20℃ immediately. If Enzyme Mix is leaved at room temperature for a long time, its activity is reduced	Store Enzyme Mix at -20℃, must be kept on ice during the preparation of reaction Mixture, and store at -20℃ immediately.

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ISSUE 2. Amplification in the negative control (without template) and no positive detection of downstream experiment (ex. PCR)

Possible cause	Comments and Suggestions
When WGA reaction is prolonged, DNA is generated during WGA reaction by random amplification of primer dimer	High-molecular weight could be generated by random amplification of primer dimer. But this resulting DNA will not affect the downstream PCR assay. Do not incubate for a long time to produce high-yield WGA product from low-amount of template. In this case, recommend to increase the amount of template DNA and incubate for a short time (incubation time recommended in protocol).

ISSUE 3. Amplification in the negative control (without template) and the positive detection of downstream experiment (ex. PCR)

Possible cause	Comments and Suggestions
Contamination of DNA template during the WGA	Decontaminate all equipment used in the WGA reaction or use the sterile equipment (e.g. barrier pipette tip). If possible, work in the separated area such as a laminar-flow hood. Recommend to store separately as the aliquot of template DNA and in the separate location.

Products

Cat. No.	Products	Size
HGA001-50	HelixAmp™ Genome Amplifier (WGA)	50 rxns
HGA001-100	HelixAmp™ Genome Amplifier (WGA)	100 rxns

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