EZassay

PfAgo Endonuclease Enhanced v2 Instructions

info@ezassay.com

www.ezassay.com

EZassay Biotechnology Ltd.

Catalog Code: PF-AGO-50
PF-AGO-500

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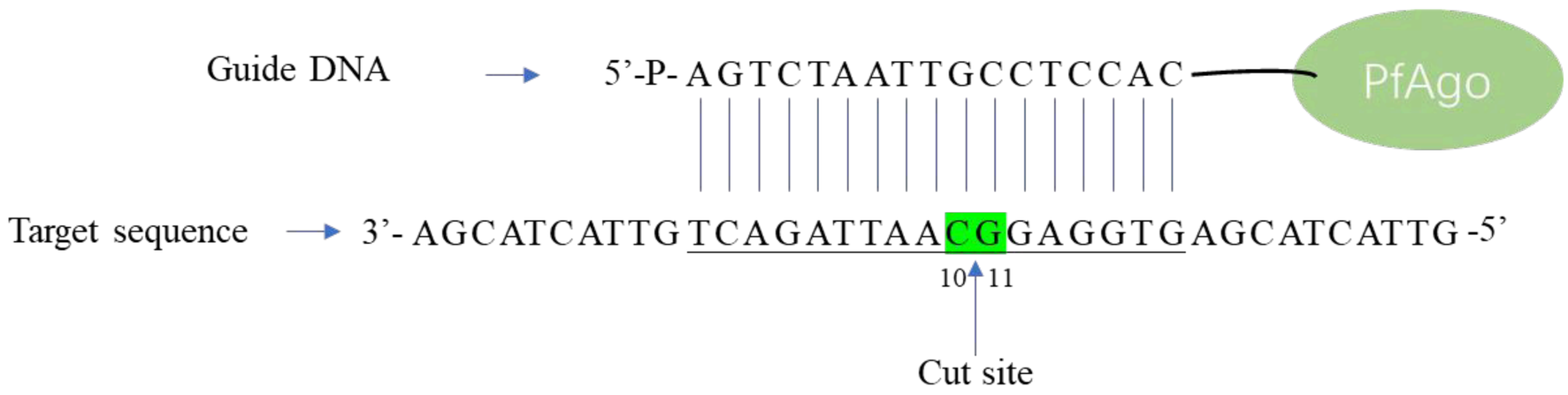
Product Information	
Product name	PfAgo Endonuclease Enhanced v2
Expression system	Escherichia coli
Quality	Recombinant protein
Form	Liquid

Product Introduction

PfAgo is an Argonaute protein derived from the thermophilic archaeon Pyrococcus furiosus, and it possesses programmable endonuclease activity. It uses 5’ -phosphorylated single-stranded DNA guides (gDNA) to recognize and cleave complementary DNA target sequences. The gDNA is typically a 16–18 nucleotide long single-stranded DNA with a 5’ -phosphate modification. Cleavage occurs between the 10th and 11th nucleotides from the 5’ end of the sequence that is complementary to the guide.



Storage

-20℃. Suggest to aliquot after receiving. Avoid repeated freeze-thaw.

Materials supplied

Cat:	PF-AGO-50	PF-AGO-500
PfAgo (1μM)	200μL*1	200μL*10
PfAgo Reaction Buffer (10X)	100μl * 1	100μl * 10
MnCl2 (40mM)	50μL * 1	50μL * 10
Positive control*	80μL	80μL* 10

Key Features of PfAgo

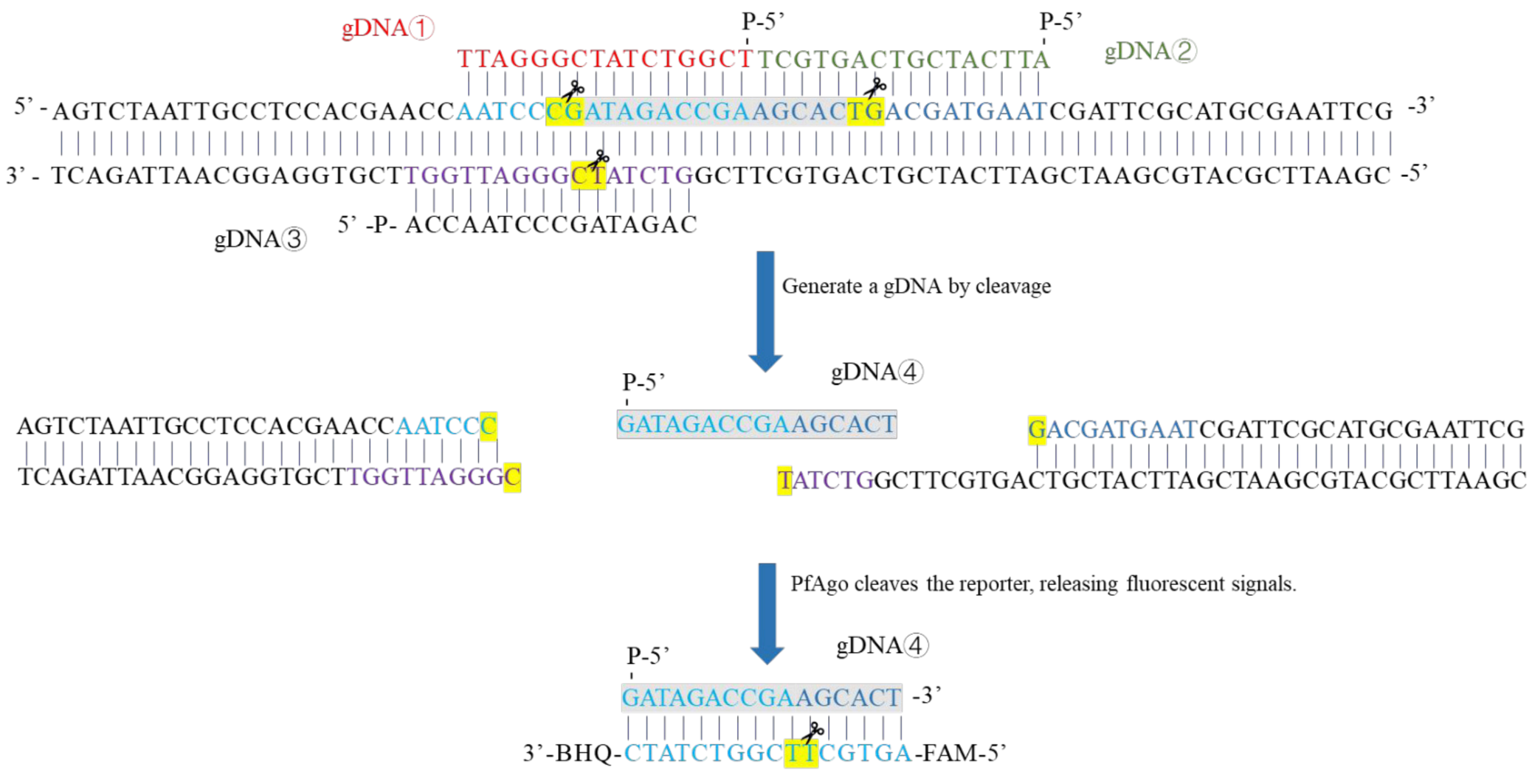
1. High-temperature activity (optimal temperature around 95°C)
2. No requirement for a PAM sequence (unlike CRISPR systems)
3. Precise target recognition guided by DNA
4. Capable of cleaving both double-stranded and single-stranded DNA
5. Suitable for multiplex detection, molecular diagnostics, and related applications

Reagents required but not provided

1. Guide DNA: Recommended gDNA length is 16–22 nucleotides, and should not exceed 31 nucleotides. When the gDNA binds to the 3' end of the target sequence, avoid having a 5' overhang on the gDNA.
2. Reporter (Optimal)
3. Thermal cycler or heat block

Application Example

PfAgo can be engineered as a highly specific nucleic acid detection tool, capable of cleaving target DNA or RNA under isothermal conditions. When combined with isothermal amplification methods such as LAMP or RPA, its sensitivity can be significantly enhanced. This makes it suitable for applications such as HPV detection, SNP genotyping, and mutation analysis.



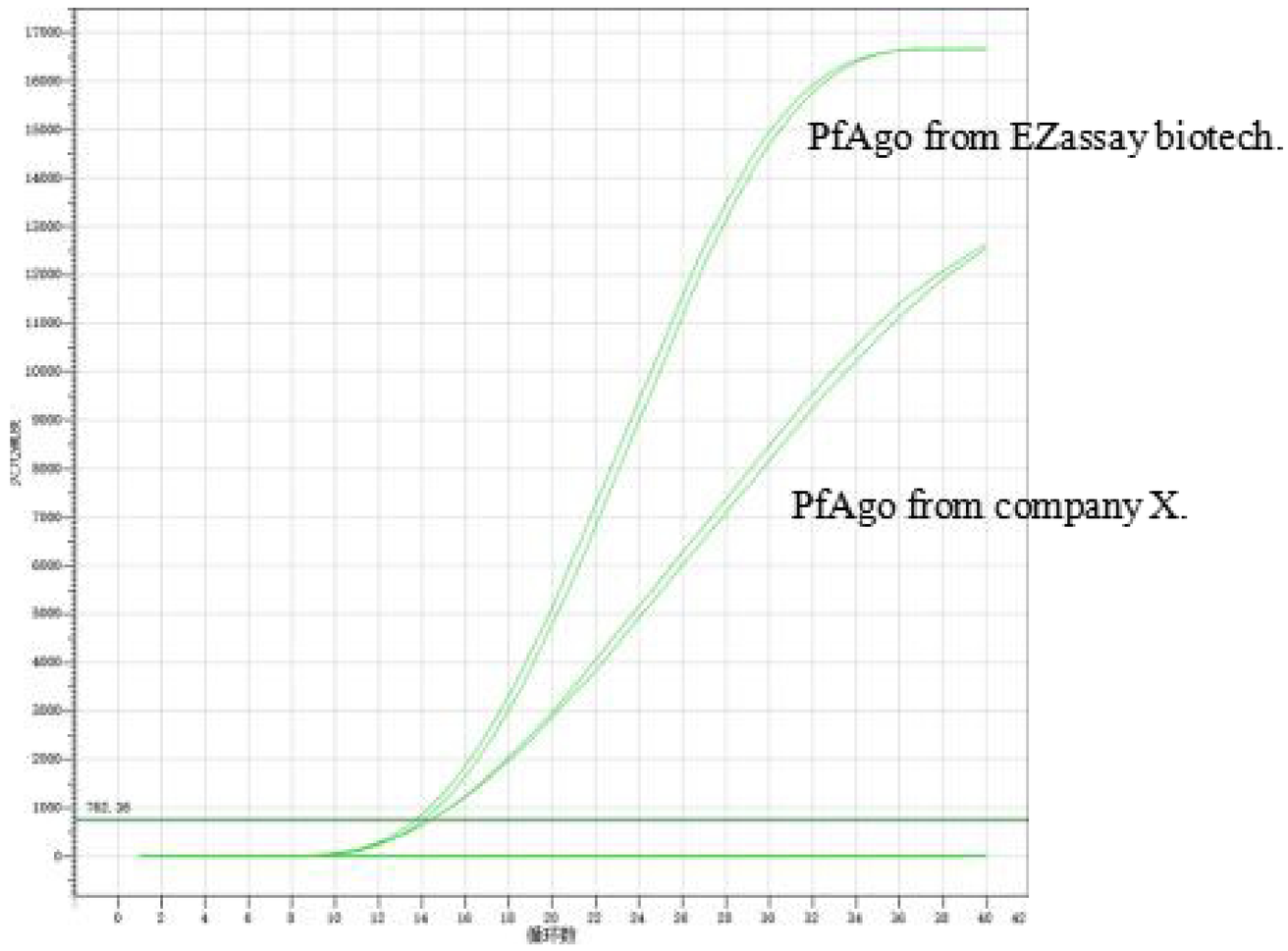
1. Amplification of the target sequence can be achieved using RPA, LAMP, or PCR.
2. Prepare the reaction mixture according to the table below:

Component	Volume	Working concentration
PfAgo Reaction Buffer (10X)	2μL	1X
MnCl2 (40mM)	1μL	2mM
gDNA (400nM)	1μL	20nM
PfAgo (1μM)	4μL	200nM
Reporter (10μM)	1μL	500nM
dsDNA*	X μL	-
H2O	Up to 20 μL	-

* dsDNA can be obtained by PCR amplification or chemical synthesis. For the positive control group, add 8 μL Positive Control. It contains gDNA, reporter, and dsDNA.

3. Vortex and briefly centrifuge.
4. Set the reaction temperature to 95°C, record fluorescence values once per minute, and run the reaction for 30–40 minutes.

Product Comparison



Experience Sharing

gDNA design can be customized. The number of strands (one, two, three, or more) should be tailored to the needs of the assay.

How to Determine the Optimal gDNA Working Concentration?
Step 1: Fix the concentrations of gDNA-1 and gDNA-2 at 20 nM each. Perform a gradient test for gDNA-3 across a range of 20-2000 nM to determine its optimal concentration, which was found to be 50 nM.
Step 2: Fix the concentrations of gDNA-1 at 20 nM and gDNA-3 at 50 nM. Screen gDNA-2 across a gradient of 20-2000 nM to determine its optimal concentration, which was found to be 20 nM.
Step 3: Fix the concentrations of gDNA-2 at 20 nM and gDNA-3 at 50 nM. Perform a gradient test for gDNA-1 from 20-2000 nM to determine its final optimal concentration.