FISHER MOLECULAR BIOLOGY

GC-PLATINUM POWER TAQ 2X MASTER MIX (Blue Dye) FS-T-1641-5

Description

GC-Platinum POWER TAQ 2X Master MIX is a ready-to-use and complete system for rapid, consistent, and accurate amplification of long PCR products (>5~20 kb). This kit optimized for PCR amplification of genomic DNA templates up to 10 kb and lambda DNA up to 20 kb. With its enhanced processivity, yield, speed and excellent 3'→5' exonuclease and 3'→5' proofreading activity, this enzyme is able to consistently deliver accurate and reliable amplification of long templates. This product is the ideal choice for long DNA templates unable to be amplified in conventional PCR, and is highly suitable for multiple downstream applications including complex cloning and genotyping experiments. The PCR product amplified with this mixture has one A added at 3'-end, so the product can be directly used for TA cloning.

This Master Mix contains blue loading dye, therefore the PCR reactions can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

Kit Contents

Contents	FS-T-1641-5	FS-T-1641-25
GC- Platinum Power TAQ 2X Master Mix (Blue dye)	5 ml	25 ml

1 ml= 40 Reactions (50 µl volume)
1 ml= 80 Reactions (25 µl volume)

Applications:

- Long range PCR
- High-fidelity PCR and primer-extension reactions
- Genotyping
- Library construction
- High GC amplification
- Next-generation DNA sequencing
- TA cloning

Source: The DNA Polymerase gene was induced and expressed in E.coli and obtained by separation and purification.
Thermal inactivation: No
5'-3' exonuclease activity: No
3-'5' exonuclease activity: Yes
Fast: The amplification speed for simple template is 5-10 s/kb, for complex template is 20-30 s/ kb

Note

Do not contaminate the GC-Platinum POWER TAQ 2X Master Mix with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the GC-Platinum Power TAQ 2X Master Mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.

Reaction Conditions

Component	25 µl	50 µl	Final
GC-Platinum POWER TAQ 2X Master Mix (Blue Dye)	12,5 µl	25 µL	1X
10µM Forward Primer	0.5 µL	1 µL	0.2 µM
10µM Reverse Primer	0.5 µl	1 µL	0.2 µM
Template DNA*	Variable	Variable	<300 ng
Water, RNase-Free	up to 25 µl	up to 50 µl	N/A

*High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed below.
Note: The optimal reaction concentration varies with different DNA templates. See table below.

Recommended PCR Program

Step	Temp (°C)	Time	Cycles
Initial Denaturation	98	45s	1
Denaturation	98	10 sec.	30
Annealing	55 ~65	30 sec.	
Extension	72	20-30 s/kb	
Final Extension	72	5 min.	1
Hold	4-12°C	∞	1

1. Template:

High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed below
(For a 50µL reaction).

DNA TEMPLATE

DNA	INPUT Amount
Plants, animals and human gDNA	10 ng~100 ng
E.coli, lambda gDNA	500 pg-200 ng
Plasmid DNA	1 pg~10 ng

Note: If the DNA template is obtained from a cDNA synthesis reaction, the template volume should be less than 10% of the total reaction volume. If long fragments are amplified, the amount of template input should be increased appropriately

2. Primers :

Oligonucleotide primers are typically 20-40 nucleotides in length with a **GC content of 40-60%**. Primers can be designed and analyzed using software such as Primer 3 The final concentration of each primer in the PCR reaction system should be in the range of 0.1-1 µM.

3. Denaturation:

98°C pre-denaturation for 45 s can fully denature most DNA templates. In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation.
Generally, the recommended denaturation condition for low-complexity DNA templates is 98℃, 5-10 s

4. Annealing:

The annealing temperature of Power TAQ 2x Master Mix is usually higher than other PCR polymerases.
Generally, primers longer than 20 nt are annealed at (lower primer Tm+3)°C for 10-30 s;
When the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm. When using a new primer set for PCR reaction, we recommend a gradient PCR to determine the optimal annealing temperature. In a two-step amplification protocol, the annealing temperature should be set to the extension temperature.

5. Extention:

The recommended extension temperature is 72 ℃ . The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension condition is 10 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 20-30 s/kb. In some cases, the extension time for cDNA templates should be less than 1 min/kb

6. Cycles:

To obtain enough yield of PCR products, 25-35 cycles are recommended.

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Storage Conditions

Store all components at -20°C in a non-frost-free freezer.