CERTIFICATE OF ANALYSIS

Product Name: **Amamus Universal PCR Master Mix (2X)**

Cat. No.: PCHPC0020

Lot. No.:

Manufacturing Date:

Expiration Date:

Optimal Storage Temp: - 20℃ ± 5℃. Avoid repeated freeze/thaw cycles

Quantity 100 Reactions

Composition (1X): Reaction buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP (dATP, dTTP, dCTP, dGTP), 0.1 U/uL Amamus Taq DNA polymerase.

Parameter	Specifications	Results
Appearance	Clear, colorless	Corresponds
Functional Testing	Amplification of 498 bp Lambda DNA fragment in the PCR using 1 pg Lambda DNA.	Corresponds
E.coli genomic DNA Contamination	≦ 0.1 pg/200U	Corresponds
Protein Purity	≧ 90%	Corresponds

Signature of Quality Assurance Supervisor, Date

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QUALITY CONTROL ASSAY

- ✓ **Functional Testing:** A 25 µl reaction in 1X Amamus Universal PCR Master Mix and 0.2 µM primers containing 1 pg Lambda DNA for 30 cycles of standard PCR amplification results in the expected 498 bp product.

- ✓ ***E.coli* genomic DNA Contamination**: 200 units Amamus Taq DNA polymerase denatured and assessed using QuantStudio Absolute Q Digital PCR System, (ThermoFisher), for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

- ✓ **Protein Purity Assay:** Amamus Taq DNA polymerase is ≧90% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Protocol for 2X Amamus Universal PCR Master Mix

I. General Guidelines:

1. **Primers:** Oligonucleotide primers are generally 15-40 nucleotides in length and ideally have a GC content of 40-60%. Final concentration of each primer in a reaction may be 0.05-1 µM.

2. **Template:** It is important that the DNA template is suitable for use in PCR in terms of purity and concentration. In addition, the template must be devoid of any contaminating PCR inhibitors (e.g. EDTA). Use of high quality, purified DNA templates greatly enhance the success of PCR. Recommended amounts of DNA templates for a 25 µl reaction are as follows:
   - Genomic DNA : 1 ng – 1 µg
   - Plasmid or viral : 1pg – 1 ng

3. **Mg^{2+} concentration:** The final Mg^{2+} concentration in 1x Amamus Universal PCR Master Mix is 2 mM. In the majority of qPCR conditions this is optimal for the Taq DNA polymerase. If necessary, Mg^{2+} and further optimized 0.5 or 1.0 mM increments using $MgCl_2$, and we suggest titrating the $MgCl_2$ to a maximum of 5 mM.

II. Prepare the PCR reaction mix:

1. Thaw the reagents and purified DNA on ice, then vortex to mix.
2. Prepare the PCR Reaction Mix in an appropriately-sized microcentrifuge tube according to the following table.

Component	volume /rxn		Final conc.
Amamus Universal PCR Master Mix (2X)	12.5	µl	1X
10 µM forward primer	0.5	µl	0.2 µM
10 µM reverse primer	0.5	µl	0.2 µM
Template DNA	variable		< 1 µg
Nuclease-free water	Up to 25	µl	-

3. Vortex to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

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III. Set up and run the PCR instrument

1. See the appropriate instrument user guide for detailed instructions to program the thermal cycling conditions, run the tube, or calibrate procedures.
2. Setup PCR program

   The master mix is compatible with typical cycling parameters. When primers with annealing temperatures above 60℃ are used, a 2-step thermocycling protocol is possible.

- **Typical Cycling Parameters (3-step PCR):**

Temperature	Time	Cycle
95℃	3 min	1
95℃	15-30 sec	30*
45-72℃	15-60 sec	
72℃	1 min/kb	
72℃	5 min	1

- **Fast Cycling Parameters (2-step PCR):**

Temperature	Time	Cycle
95℃	3 min	1
95℃	15-30 sec	30*
60-72℃	1 min/kb	
72℃	5 min	1

* Generally, 30 cycles result in optimal amplification of desired products. Occasionally, up to 35 cycles may be performed, especially for detection of low-copy targets.

3. Load the tubes into the PCR instrument.
4. Start the run.