TaqPath™ ProAmp™ Master Mixes

Genotyping and copy number variation PCR workflows

Pub. No. 100040972 Rev. D

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqPath™ ProAmp™ Master Mixes User Guide* (Pub. No. MAN0015758). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of TaqPath™ ProAmp™ Master Mix and TaqPath™ ProAmp™ Multiplex Master Mix. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqPath™ ProAmp™ Master Mixes User Guide* (Pub. No. MAN0015758).

TaqPath™ ProAmp™ Master Mix is formulated with ROX™ dye (absorption 575 nm, emission 602 nm). TaqPath™ ProAmp™ Multiplex Master Mix is formulated with MUSTANG PURPLE™ dye (absorption 647 nm, emission 654 nm).

Both formulations contain heat-labile uracil-DNA glycosylase (UNG) and dUTP. Heat-labile UNG degrades any carryover PCR products, helping to prevent downstream contamination and possible false positives. The heat-labile enzyme is active at room temperature and is completely inactivated during the initial ramp to the 95°C hold step.

Procedural guidelines

Guidelines for DNA template input

- All wells that use the same assay must contain similar amounts of sample DNA.
- For genotyping experiments, use a final DNA concentration of at least 0.2 ng/µL for each reaction.
- For copy number experiments, use a final purified DNA concentration of 1 ng/µL for each reaction.

Guidelines for PCR reactions

- For copy number experiments, we recommend four replicates of each reaction.
- Reactions can be prepared depending upon experimental requirements. Scale the components according to the number of reactions and include 10% overage.
- For reaction volumes that are different from those detailed, scale all components proportionally and include 10% overage.
- For genotyping experiments, no template control (NTC) reactions are required to call the genotype. NTC reactions can also be used to identify PCR contamination.

  NTC reactions contain all reaction components (master mix, assays, water) except DNA sample.

Set up the PCR reactions

1. Prepare the required number of reactions according to the appropriate table, plus 10% overage.

Table 1 PCR reactions for genotyping experiments

Component	Volume per reaction		
	5 µL	**10 µL**	**25 µL**
TaqPath™ ProAmp™ Master Mix	2.5 µL	5.0 µL	12.5 µL
TaqMan™ SNP Genotyping Assay[1] (20X)	0.25 µL	0.5 µL	1.25 µL
Genomic DNA *-or-* NTC	up to 2.25 µL	up to 4.5 µL	up to 11.25 µL
Nuclease-Free Water	to 5 µL total	to 10 µL total	to 25 µL total
Total volume	**5 µL**	**10 µL**	**25 µL**

[1] For Research Use Only. Not for use in diagnostic procedures.

Table 2 PCR reactions for copy number experiments

Component	Volume per reaction	
	10 µL	**20 µL**
TaqPath™ ProAmp™ Master Mix	5.0 µL	10.0 µL
TaqMan™ Copy Number Assay[1] (20X)	0.5 µL	1.0 µL
TaqMan™ Copy Number Reference Assay[1] (20X)	0.5 µL	1.0 µL
Genomic DNA *-or-* NTC	up to 4 µL	up to 8 µL
Nuclease-Free Water	to 10 µL total	to 20 µL total
Total volume	**10 µL**	**20 µL**

[1] For Research Use Only. Not for use in diagnostic procedures.

2. Mix the components thoroughly, then centrifuge briefly to spin down the contents and eliminate air bubbles.
3. Add the appropriate volume of each reaction to each well of an optical plate.
4. Seal the plate with an optical adhesive cover, then centrifuge briefly to spin down the contents and eliminate air bubbles.

Store the reaction plate for up to 72 hours at room temperature.

Set up and run the real-time PCR instrument

1. Load the reaction plate in the real-time PCR instrument.
2. Set the appropriate experiment settings and PCR thermal cycling conditions.

   Note: Heat-labile UNG is active during the reaction setup and is completely inactivated during the first ramp to the 95°C hold step.

Table 3 Genotyping experiments: standard cycling

Step	Temperature	Time	Cycles
Pre-Read	60°C	30 seconds	Hold
Initial denature / Enzyme activation	95°C	5 minutes	
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	60 seconds[1]	
Post-Read	60°C	30 seconds	Hold

[1] For Drug Metabolizing Enzyme (DME) assays, set duration to 90 seconds.

Table 4 Genotyping experiments: fast cycling

Step	Temperature	Time[1]	Cycles
Pre-Read	60°C	30 seconds	Hold
Initial denature / Enzyme activation	95°C	5 minutes	
Denature	95°C	5 seconds	40
Anneal / Extend	60°C	30 seconds	
Post-Read	60°C	30 seconds	Hold

[1] Optional cycling for Fast 96-well or 384-well plates only.

Table 5 Copy number experiments: standard cycling

Step	Temperature	Time	Cycles
Initial denature / Enzyme activation	95°C	10 minutes	Hold
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	60 seconds	

3. Set the reaction volume appropriate for the reaction plate.
4. Start the run.

Perform allelic discrimination

For genotyping experiments, perform allelic discrimination using a post-read temperature of 60°C.

See the instrument user guide for further information.

Analyze the results

Data analysis varies depending on the instrument.

See the *TaqPath™ ProAmp™ Master Mixes User Guide* (Pub. No. MAN0015758) and the instrument user guide.

Ordering information

Table 6 TaqPath™ ProAmp™ Master Mix formulations

TaqPath™ ProAmp™ Master Mix with ROX™	TaqPath™ ProAmp™ Multiplex Master Mix with MUSTANG PURPLE™	Amount
A30865	A30868	1 × 1 mL
A30866	A30869	1 × 10 mL
A30871	A30873	2 × 10 mL
A30867	A30870	1 × 50 mL
A30872	A30874	2 × 50 mL

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. 100040972

Revision	Date	Description
D	20 January 2022	Change manufacturer to Austin, TX.
C	04 September 2018	Change manufacturer to Vilnius
B	11 July 2016	Change multiplex master mix name; add final DNA concentration guidelines; add UNG and DME assays information; add pre- and post-read steps to genotyping thermal cycling conditions
A	April 2016	New document.