



# ampliTune® qPCR Probe Mix (5X) with ROX

Cat. No.	Pack Size	Conc. (MgCl <sub>2</sub> )
12025	0.1 ml SAMPLE	15 mM
12250	1 ml	15 mM

## Description

ampliTune® qPCR Probe Mix has been designed to deliver superior performance compared to standard master mixes with impressive sensitivity and accuracy even with challenging targets. The all-in-one master mix includes all components necessary to perform the reaction - only template, primers and probes needs to be added. ampliTune® polymerase is supplied in a proprietary reaction buffer that enables efficient amplification of regular and GC-rich targets. A 12 min incubation step at 95°C activates the polymerase and prevents primer dimer formation and non-specific amplification during qPCR.

## Advantages

- ✓ Suitable for single and duplex assays
- ✓ Superior specificity and performance with AT-rich and GC-rich templates
- ✓ Increased sensitivity for low copy number targets
- ✓ UNG treatment capability to prevent carryover contamination from previous runs<sup>1</sup>
- ✓ Stable at room temperature for up to 1 month
- ✓ Benchtop stability for 48 hours for pre-assembled reactions

<sup>1</sup>UNG is not included and must be purchased separately.

## Storage & Shipping

Store at -20°C, shipping at room temperature. Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of ampliTune® qPCR Probe Mix.

## Reagent composition

ampliTune® DNA polymerase is supplied in a 5X qPCR buffer, dNTPs including dUTP, 15 mM MgCl<sub>2</sub> (1x PCR solution – 3 mM MgCl<sub>2</sub>) and internal reference based on ROX. 100% DMSO is supplied in a separate vial.

DMSO is a PCR enhancer that increases the specificity and yield of PCR. DMSO is recommended as a PCR additive to increase the heat lability of GC-rich templates and it is also required to relax secondary structures. While testing it is recommended to include one sample with additional 2.5 % DMSO to test if it improves the results. The highest DMSO concentration recommended is 10% which should be utilized for templates with GC content over 70%.

## Addition of 100% DMSO

Final conc. of DMSO	20 µl/rxn
2.5 %	0.5 µl
5 %	1 µl
10 %	2 µl

## Recommended qPCR reaction mix

Component	Volume	Final conc.
ampliTune® qPCR Probe Mix	4 µl	1X
Primer Forward (10 pmol/µl)	0.4-0.8 µl	0.2-0.4 µM
Primer Reverse (10 pmol/µl)	0.4-0.8 µl	0.2-0.4 µM
Probe	x µl	0.1-0.25 µM
Optional UNG	x µl	x U/ µl
Optional 100% DMSO	variable	up to 10%
DNA template	variable	variable <sup>2</sup>
H <sub>2</sub> O PCR grade	up to 20 µl	
<b>Total</b>	<b>20 µl</b>	

<sup>2</sup>cDNA 0.1 pg/µl – 10 ng/µl; gDNA 10 pg/µl – 4 ng/µl

## Recommended qPCR cycles

Cycle step	Temp.	Time	Cycles
Optional UNG treatment	50 °C	2 min	1
Initial activation	95 °C	12 min	1
Denaturation	95 °C	15-20 s	} 40
Annealing/ Elongation	60 °C	60 s <sup>3</sup>	

<sup>3</sup>Use 30 sec for annealing and elongation for templates shorter than 150 bp.

## Product use limitation

This product is developed and supplied for research purposes and *in vitro* use only. Please refer to www.selectbiotech.com for Material Safety Data Sheet of the product.

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## CERTIFICATE OF ANALYSIS

### Endodeoxyribonuclease activity

No contaminating endonuclease activity was observed upon incubation of 10 µl of ampliTune® qPCR Probe Mix (5X) with 1 µg of pUC19 DNA in 50 µl of reaction mixture for 4 hours at 37°C.

### Exodeoxyribonuclease activity

No contaminating exonuclease activity was detected after incubation of 10 µl of ampliTune® qPCR Probe Mix (5X) with 1 µg of digested DNA in 50 µl of reaction mixture for 4 hours at 37°C.

### Ribonuclease activity

No contaminating RNase activity was detected after incubation of 1 µg of RNA transcript with 10 µl of ampliTune® qPCR Probe Mix (5X) for 4 hours at 37°C.

### Functional assay

ampliTune® qPCR Probe Mix (5X) was tested for amplification of 75, 196 and 343 bp DNA fragments.

Quality authorized by: Mgr. Petra Mazancova, PhD. 

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