



# Product Manual

## **AMPIGENE<sup>®</sup> qPCR Probe Mix Lo-ROX**

Catalog #: ENZ-NUC105

ENZ-NUC105-0200 for 200 rxns

ENZ-NUC105-1000 for 1000 rxns



# Product Manual

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Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions.



Please contact Enzo Life Sciences Technical Support if necessary.

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## DESCRIPTION

Combined with the latest advancements in polymerase technology and advanced buffer chemistry AMPIGENE<sup>®</sup> qPCR Probe Mix offers market leading performance with minimal optimization.

Enzo Life Sciences' real-time PCR probe mixes have been designed for use on a wide range of probe technologies including Taqman<sup>®</sup>, Molecular Beacons<sup>®</sup> and Scorpion probes<sup>®</sup>.

AMPIGENE<sup>®</sup> qPCR Probe Mix uses proprietary small molecular inhibitor technology that prevents formation of primer-dimers to improve reaction sensitivity and specificity.

High-throughput screening has resulted in a buffer system that allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions.

Pack Size	Format	
200 x 20µl rxns	2x AMPIGENE <sup>®</sup> qPCR Probe Mix Lo-ROX	2 x 1ml
1000 x 20µl rxns	2x AMPIGENE <sup>®</sup> qPCR Probe Mix Lo-ROX	10 x 1ml



Protect from prolonged exposure to light.

## SHIPPING AND STORAGE

On arrival the kit should be stored at -20°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

## INSTRUMENT COMPATIBILITY

Manufacturer	Instrument	Lo-ROX	Hi-ROX
Analytica Jena	qTower	Yes	Yes
Applied Biosystems	7500, 7500 FAST, Viiia7 <sup>™</sup>	Yes	No
Applied Biosystems	7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne <sup>™</sup> , StepOne <sup>™</sup> Plus	No	Yes
Bio-Rad <sup>®</sup>	iCycler <sup>®</sup> , MyiQ <sup>®</sup> , iQ <sup>™</sup> 5, Opticon <sup>™</sup> , Opticon <sup>™</sup> 2, Chromo4 <sup>™</sup> , MiniOpticon <sup>™</sup> , CFX96 <sup>™</sup> , CFX384 <sup>™</sup>	Yes	No
Cepheid <sup>®</sup>	Smartcycler <sup>®</sup>	Yes	Yes
Eppendorf	Mastercycler <sup>®</sup> ep realplex, Mastercycler <sup>®</sup> realplex 2S	Yes	Yes
Illumina <sup>®</sup>	Eco <sup>™</sup>	Yes	Yes
Qiagen/Corbett	Rotor-Gene <sup>™</sup> 3000, 6000, Q	Yes	Yes
Roche Applied Science	Lightcycler <sup>®</sup> 480, Lightcycler <sup>®</sup> Nano	Yes	Yes
Stratagene (Agilent)	MX 4000P <sup>®</sup> , MX 3000P <sup>®</sup> , MX 3005P <sup>®</sup>	Yes	No
Takara	Cycler Dice <sup>®</sup>	Yes	Yes
Techne	Quantica <sup>®</sup>	Yes	Yes

## IMPORTANT CONSIDERATIONS

**Primer design:** For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80bp and 200bp. With all manufacturers master mixes the shorter the amplicon length the faster the reaction can be cycled. Amplicon lengths should not exceed 400bp. Primers should have a predicted melting temperature of around 60°C. For taqman probes choose probe close to 5' primer, avoid terminal guanosine residues.

## REACTION SETUP

1. Briefly vortex 2x AMPIGENE<sup>®</sup> qPCR Probe Mix Lo-ROX.
2. Prepare a master mix based on the following table:

Reagent	20µl reaction	Final concentration	Notes
2x AMPIGENE <sup>®</sup> qPCR Probe Mix Lo-ROX	10µl	1x	
Forward primer (10µM)	0.8µl	400nM	See above for optimal primer design
Reverse primer (10µM)	0.8µl	400nM	
Probe (10µM)	0.4µl	200nM	
Template DNA	<100ng cDNA, <1µg genomic	variable	See above for template considerations
PCR grade dH <sub>2</sub> O	Up to 20µl final volume		

3. Program the instrument using following conditions, acquiring data on the appropriate channel:

Cycles	Temperature	Time	Notes
1	95°C	2min	Polymerase activation, 2 minutes for cDNA and 3 minutes for genomic
40	95°C 60°C to 65°C	5 seconds 20-30 seconds	Denaturation Anneal/Extension (do not exceed 30 seconds, do not use temperatures below 60°C)
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridization probes only.



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