Product Manual

AMPIGENE® qPCR 1-Step Green Kit Hi-ROX

Catalog #: ENZ-NUC109

ENZ-NUC109-0200 for 200 rxns

ENZ-NUC109-1000 for 1000 rxns

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON- INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

FOR RESEARCH USE ONLY.

THE PRODUCT MAY BE USED ONLY FOR *IN VITRO* RESEARCH PURPOSES.

Product Manual

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.

TABLE OF CONTENTS

DESCRIPTION

Enzo Life Sciences' AMPIGENE® qPCR 1-Step Green Kit uses the latest developments in reverse transcriptase technology and buffer chemistry for efficient cDNA synthesis and PCR in a single tube.

Our modified MMLV reverse transcriptase (RTase) is both thermostable and extremely active. The enzyme is blended with RNase inhibitor preventing degradation of RNA by contaminating RNase. The RTase is not inhibited by ribosomal and transfer RNAs, total RNA is an ideal substrate.

Enzo Life Sciences' Green Mixes use an intercalating dye which does not inhibit PCR, unlike other popular dyes.

AMPIGENE® qPCR 1-Step Green 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.

Component	200 reactions	1000 reactions
2x AMPIGENE® qPCR 1-Step Green Mix Hi-ROX	2 x 1ml	10 x 1ml
20x RTase with RNase inhibitor	2 x 200µl	10 x 200µl

SHIPPING AND STORAGE

Protect from prolonged exposure to light.

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, Viia7™	Yes	No
Applied Biosystems	7000, 7300,7700,7900, 7900HT, 7900HT FAST, StepOne™, StepOne™ Plus	No	Yes
Bio-Rad®	iCycler®, MyiQ®, iQ ™5, Opticon™, Opticon™2, Chromo4™, MiniOpticon™, CFX96™, CFX384™	Yes	No
Cepheid®	Smartcycler®	Yes	Yes
Eppendorf	Mastercycler® ep realplex, Mastercycler® realplex 2S	Yes	Yes
Illumina®	Eco™	Yes	Yes
Qiagen/Corbett	Rotor-Gene™ 3000, 6000, Q	Yes	Yes
Roche Applied Science	Lightcycler®480, Lightcycler®Nano	Yes	Yes
Stratagene (Agilent)	MX 4000P®, MX 3000P®, MX 3005P®	Yes	No
Takara	Cycler Dice®	Yes	Yes
Techne	Quantica®	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.

REACTION SETUP

1. Before starting, briefly vortex 2x AMPIGENE® qPCR 1-Step Green Mix Hi-ROX.
2. Prepare a master mix based on the following table; table, we recommend also setting up a no-RTase control

Reagent	20µl reaction	Final concentration	Notes
2x AMPIGENE® qPCR 1-Step Green Mix Hi-ROX	10µl	1x	
Forward primer (10µM)	0.8µl	400nM	See above for optimal primer design
Reverse primer (10µM)	0.8µl	400nM	
20x RTase	1.0-2.0µl	1x or 2x	1.0µl is recommended 2.0µl will improve Ct but may increase primer dimers
Template RNA	1pg to 1µg total RNA >0.01pg mRNA	variable	
PCR grade dH_2O	Up to 20µl final volume		

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

Cycles	Temperature	Time	Notes
1	45°C to 55°C	10min	Reverse transcription, 45°C is recommended for most applications, 55°C should be used only when amplicon contains regions of high secondary structure
1	95°C	2min	Polymerase activation, 2 minutes
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

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com