



AMPIGENE® Lyo-Probe 1-Step kit

REF ENZ-NUC143-2000

2000 Reactions

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

The **AMPIGENE® Lyo-Probe 1-Step Kit** is specially formulated for reliable RT-qPCR-based detection of viral RNA sequences and is ideal for the development of lyophilized diagnostic assays.

The kit includes a glycerol-free 4X qPCR mix containing hot start Taq polymerase, dNTPs, MgCl₂ and a blend of excipients to ensure reliable lyophilization, without loss of activity. **AMPIGENE® Lyo-Probe RTase** (3200X) is provided separately in a highly concentrated format, with RNase inhibitor included. The kit is suitable for lyophilization into beads or cakes.

ASSAY PRINCIPLE

Polymerase chain reaction (PCR) uses *Taq* polymerase enzyme, which directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template, by adding nucleotides to the 3' end of a custom-designed oligonucleotide annealed to the template DNA¹.

KNOWN APPLICATION

Amplification of nucleic acid targets with PCR methods.

PRODUCTS SUPPLIED

Component	2000 reactions
AMPIGENE® Lyo-Probe Mix (4X)	2 x 5 mL
AMPIGENE® Lyo-Probe RTase (3200X) (with RNase inhibitor)	1 x 12.5 µL

MATERIALS NEEDED (Not Provided)

- Target RNA
- Primers
- Thermal Cycler

STORAGE AND SHELF-LIFE

- Upon receipt, store kit at -20°C. These products are stable under these conditions up to the expiration date indicated in the vial label.
- Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months from date of receipt. 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.

PERFORMANCE CONSIDERATIONS

1. Do not use reagents past their expiration date.
2. Cross-contamination of samples could cause false results. Use care when working with more than one sample.

LIMITATIONS

- This procedure is for research use only. It is not intended for diagnostic or therapeutic use.

PRECAUTIONS

1. Refer to reagent Safety Data Sheet (SDS) from precautions.
2. Specimens, before and after fixation, and all materials exposed to them should be handled and disposed of with proper precautions.
3. Never pipette reagents by mouth and avoid contact with skin and mucous membranes with reagents and specimens. If reagents and/or specimens come into contact with sensitive areas, rinse thoroughly with water and follow your institution's safety protocols.
- 4.

TECHNICAL NOTES

For technical support and troubleshooting you can submit a technical enquiry online, call us direct, or alternatively email with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of gel images

Product Setup

Before starting, thaw and briefly vortex the bottle of 4X **AMPIGENE® Lyo-Probe Mix**.

Add 1.25 µL of 3200X **AMPIGENE® Lyo-Probe RTase** for each mL of

GLOBAL HEADQUARTERS

Enzo Life Sciences, Inc.
81 Executive Blvd, Ste 3
Farmingdale, NY 11735 USA
T 1-800-942-0430
F 1-610-941-9252
E info-usa@enzolifesciences.com
www.enzolifesciences.com

EUROPE

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lausen, Switzerland
T +41 61 926 89 89
F +41 61 926 89 79
E info-ch@enzolifesciences.com
www.enzolifesciences.com



4X AMPIGENE® Lyo-Probe Mix used, taking care as the RTase solution is viscous.

Briefly vortex to mix the components. The 4X AMPIGENE® Lyo-Probe 1-Step Mix is now ready to use or can be stored for up to 3 days at 4 °C. If less than the entire bottle is required, a smaller amount of 4X AMPIGENE® Lyo-Probe 1-Step Mix can be prepared, and the remaining separate components (4X AMPIGENE® Lyo-Probe Mix and 3200X AMPIGENE® Lyo-Probe RTase) returned to storage at between -30 °C and -15 °C.

Add primers and probes to the 4X Lyo-Probe 1-Step Mix, then start the freeze-drying cycle. We suggest diluting to 1x or 2x with these extra components and water to facilitate the lyophilization process. The mix has the following critical temperatures: onset of collapsing temperature (T_c): -35.1 °C, onset of glass transition temperature (T_g): 68.9 °C, mid-point T_g : 75.1 °C, and end point T_g : 81.3 °C.

We have tested the following conditions in 2 mL glass vials containing 500 µL of the 1-step mix. Shorter drying times will be required if standard PCR tubes and plates are used. Further custom optimization may be needed depending on the lyophilization instrument.

Stage	Step	Shelf Temp. (°C)	Time (min)	Pressure (µBar)	Description
Thermal Treatment Stage	-	+2 to +6	N/A	Atmospheric	Loading
	1	+5	10	Atmospheric	Hold
	2	-50	110	Atmospheric	Ramp (0.5°C/min)
	3	-50	180	Atmospheric	Hold
Primary Drying Stage	4	-45	10	30	Ramp (0.5°C/min)
	5	-45	5400	30	Hold
Secondary Drying Stage	6	+20	130	30	Ramp (0.5°C/min)
	7	+20	600	30	Hold
Actions at end of cycle	-	+20	N/A	Half Atmospheric	Backfill with N ₂
	-	+20	N/A	Half Atmospheric	Stopper
	-	+20	N/A	Atmospheric	Aerate

Reaction Setup

- To test the wet mix (obtained after step 3 of product setup), prepare a master mix based on the following table. We also recommend setting up a no-RTase control:

Reagent	20 µL reactions	Final Concentration
Lyo-Probe 1-Step Mix (4X)	5 µL	1X
Forward primer (10 µM)	1-2 µL	400 nM-1 µM
Reverse primer (10 µM)	1-2 µL	400 nM-1 µM
Probe (10 µM)	0.25-1 µL	125-500 nM
RNA template	2-5 µL	Variable
PCR grade dH ₂ O	Up to 20 µL final volume	

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

Step	Temp. General	Temp. SARS-CoV-2 Detection	Time	Cycles
Reverse transcription	45°C to 55°C	55 °C	5-10 minutes singleplex	1
			10-20 minutes multiplex	
Polymerase activation and RTase inactivation	95°C	95 °C	3 minutes	1
Denaturation Anneal/Extension	95 °C	95 °C	15 seconds	50
	55 °C to 65 °C	58 °C	30 seconds	

REFERENCES

- Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51 Pt 1:263-73.

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EUROPE

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lausen, Switzerland
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F +41 61 926 89 79
E info-ch@enzolifesciences.com
www.enzolifesciences.com