OmniAmp™ Polymerase: A new tool for rapid loop-mediated isothermal amplification (LAMP) of RNA and DNA targets

Lucigen Corporation
2952 Parmenter St., Middleton, WI 53562

Lucigen®
Simplifying Genomics
www.lucigen.com

ABSTRACT

RNA or DNA target detection in less than 30 minutes

PCR-based detection of infectious organisms or genetic mutations is the recognized standard for molecular diagnostics. However, the time, complexity and expense of PCR-based diagnostics have led to development of rapid test methodologies that can bring testing closer to the patient. Isothermal amplification technology can offer faster and less reagent-intensive molecular detection that requires simpler and lower cost instruments than PCR. Several isothermal amplification methods exist, but many rely on complex protocols, multiple enzymes or special reagents to perform RNA-dependent amplification. This poster describes the performance of OmniAmp™, an isothermal amplification polymerase ideally suited for loop-mediated amplification (LAMP). OmniAmp™ uniquely possesses innate reverse transcriptase activity that amplifies either RNA or DNA targets in a single-enzyme, single buffer system and provides faster time to result.

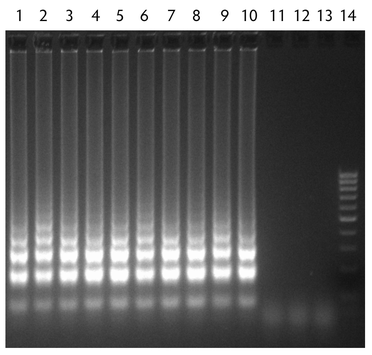
OmniAmp™ Polymerase is Ideal for Rapid Molecular Detection Tests

Property	OmniAmp Reverse Transcriptase	Bst Polymerase	Conventional Reverse Transcriptase
Theremostablility > 70°C	✓	⊘	⊘
RNA or DNA Amplification	✓	⊘	✓
Rapid Isothermal Amplification	✓	✓	⊘
Ability to be dried (lyophilized)	✓	✓	⊘

OmniAmp™ Directly Amplifies RNA or DNA Targets

DNA Target

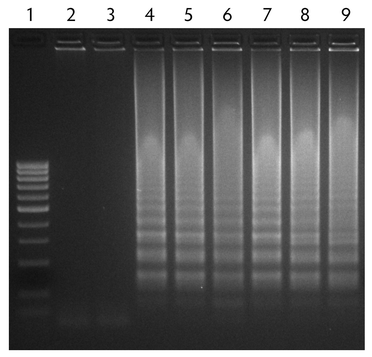
DNA LAMP Amplification



Lanes 1, 2 E. ictaluri DNA 1:10 dilution
Lanes 3, 4 E. ictaluri DNA 1:100 dilution
Lanes 5, 6 E. ictaluri DNA 1:1,000 dilution
Lanes 7, 8 E. ictaluri DNA 1:10,000 dilution
Lanes 9, 10 E. ictaluri DNA 1:100,000 dilution
Lanes 11-13 No Target Control
Lane 14 100 BP ladder

RNA Target

RT-LAMP: Reliable single-enzyme LAMP amplification directly from RNA

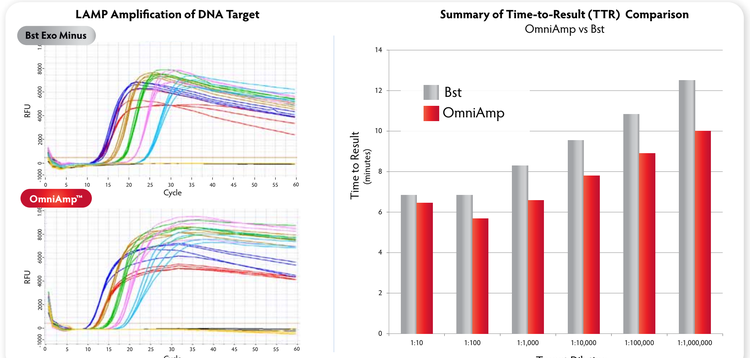


Lane 1 100 bp ladder
Lanes 2, 3 No target control
Lanes 4, 5 Target RNA, 1:10 dilution
Lanes 6, 7 Target RNA, 1:100 dilution
Lanes 8, 9 Target RNA, 1:1000 dilution

DNA and RNA LAMP reactions

Agarose gel images of LAMP Reaction from serial dilutions of target DNA or RNA. The common "ladder" banding pattern within a smear is customary of LAMP reactions. All amplifications used only OmniAmp™ polymerase. No dedicated RT step or use of additional RT enzyme was used for RNA amplification.

OmniAmp is faster than Bst in LAMP



Quantitation of LAMP Amplification from DNA Target

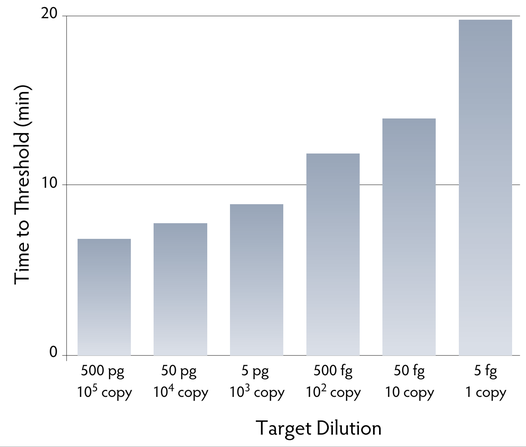
Quantitative results of LAMP amplification of E. ictaluri DNA over several orders of magnitude of target concentration.

Color key: 1:10 = Red, 1:100 = Blue, 1:1,000 = Brown, 1:10,000 = Green, 1:100,000 = Pink, 1:1,000,000 = Light Blue. 1:10,000,000 dilution (yellow) and NTC's (Black) showed no amplification.

The cycler was programmed to read amplification in 30-second intervals (cycles).

LAMP Reaction Detects Single Copy of *C. diff* DNA

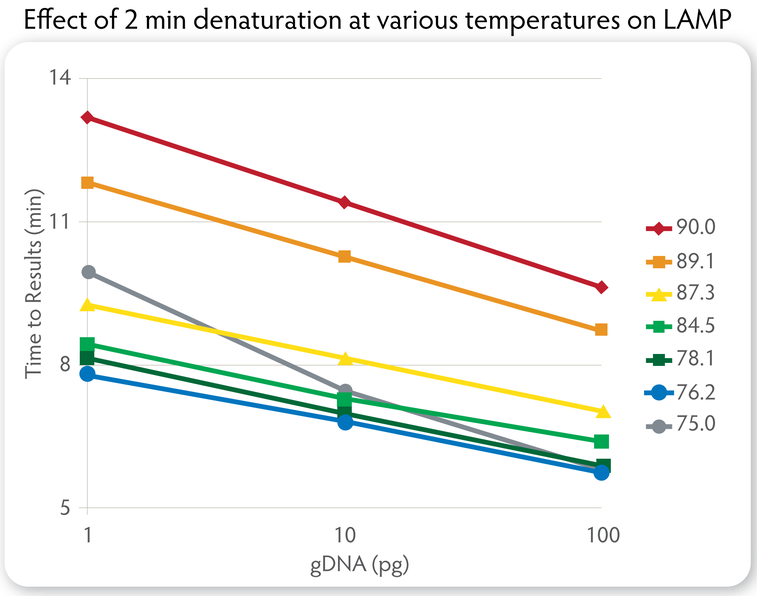
Amount	T_t (min) Mean	s.d.	n (of 3)
500 pg	6.9	0.03	3
50 pg	7.8	0.15	3
5 pg	8.9	0.23	3
500 fg	11.9	0.8	3
50 fg	14.0	1.0	3
5 fg	19.8	-	1
NTC	-	-	0



Triplicate tcdA LAMP reactions of *C. diff* DNA from 1 × 10^5 to 1 copy.

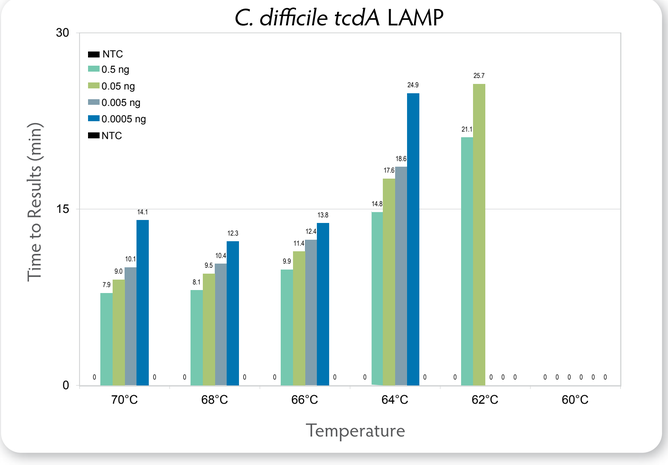
Up to 90°C Denaturation, then LAMP Reaction

Effect of 2 min denaturation at various temperatures on LAMP



Effect of high temperature incubation on enzyme activity: OmniAmp™ polymerase was incubated at indicated temperatures for 2 minutes to simulate nucleic acid denaturation conditions. After incubation, a standard LAMP assay was performed.

68 – 70°C Temperature Optimum for *C. diff* LAMP assay



Temperature optimization of *C. diff* LAMP Assay: Range of temperatures was tested across a dilution series of extracted *C diff* DNA. Time to results for all dilutions tested fell under 15 minute threshold at 70 – 66°C reaction temperature.

OMNIAMP SUMMARY

- Faster to result than Bst polymerase (LAMP)
- Single enzyme amplification of DNA or RNA using a single buffer system
- High thermostability allows use with challenging RNA or DNA clinical targets

OmniAmp™ is for Research Use Only.