HZYMES BIOTECH

LAMP/RT-LAMP

LAMP/RT-LAMP – RAPID NUCLEIC ACID DETECTION

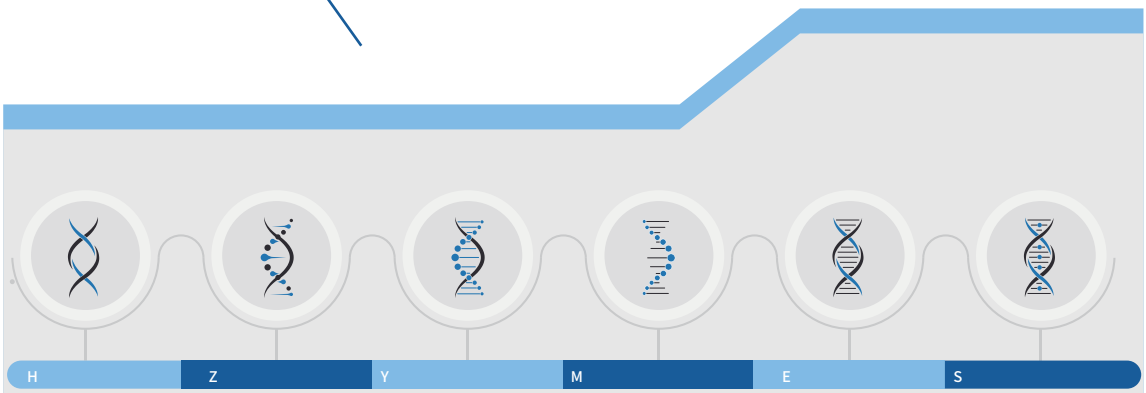
HZYMES BIOTECHNOLOGY CO.,LTD

BACKGROUND

Loop-mediated isothermal amplification(LAMP) is a versatile technique for detecting target DNA and RNA, enabling rapid molecular diagnostic assays with minimal equipment. LAMP technique does not require changing the reaction temperature and it' s extremely fast, which is making them particularly well suited for field applications and point-of-care molecular diagnostics assays.

With well-designed 4-6 primers and the specific Bst DNA polymerase, the detection of the target of interest can be done within 30 min. There are many LAMP detection methods, including the fluorescent method, colorimetric method, fluorescent-probe-based method, turbidity method, HNB, etc. The LAMP solution provided by Hzymes is able to help achieve high sensitivity, good primer compatibility, and excellent performance in specificity, tolerance, and stability.

OUR ADVANTAGES



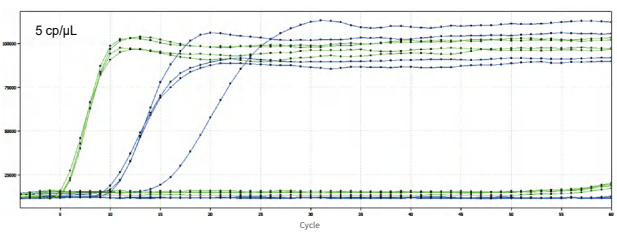
H	Z	Y	M	E	S
Fast Reaction	**High Sensitivity**	**High Specificity**	**Lyophilization**	**Inhibitor Tolerance**	**Good Primer Compatibility**
< 25 min	< 1 cp/μL	No amplification with NTC samples	Lyophilized beads and in-situ lyophilization	Extraction-free LAMP/RT-LAMP	Compatible with different DNA/RNA targets

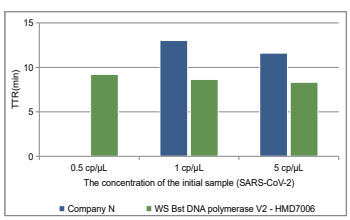
BST DNA POLYMERASE V2

Bst DNA polymerase V2 is derived from Bacillus stearothermophilus DNA Polymerase I, which contains 5´→3´ DNA polymerase acBvity and strong strand displacement activity but lacks 5´→3´ exonuclease activity. It can be used for DNA strand displacement reaction and isothermal amplification. Hzymes Biotech' s Bst DNA polymerase V2 has fast amplification speed, strong resistance to PCR inhibitors, and good sample compatibility. In addition, the WS Bst DNA polymerase V2 is modified by aptamers, which can effectively prevent false positive results. The glycerol-free Bst DNA Polymerase V2 version is more suitable for developing lyophilized LAMP kits.

Fast polymerization

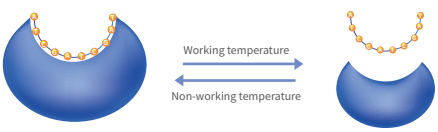
Blue:Company N Green:HMD7006 - WS Bst DNA polymerase V2



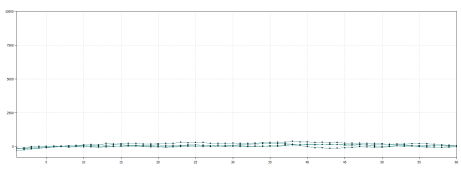


Polymerization speed is a crucial indicator for evaluating Bst DNA polymerase. The SARS-CoV-2 N gene target(0.5 cp/μL, 1 cp/μL, and 5 cp/μL)can be successfully detected using WS Bst DNA polymerase V2(HMD7006). And the whole detection performs faster than company N at the same template concentration(5 cp/μL).

Specificity

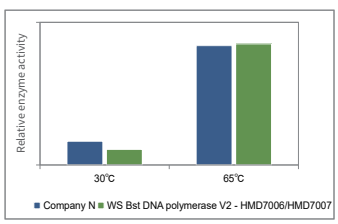


Hzymes Biotech's WS Bst DNA polymerase V2(HMD7006 and HMD7007) uses aptamer-based blocking technology. It blocks the enzyme's activity through the tight bond of the aptamer and the polymerase at a lower temperature. But at the working temperature, the binding of the aptamer to the enzyme is released, and the enzyme becomes active.



Example: NTC diagram when detecting SARS-CoV-2 N gene target

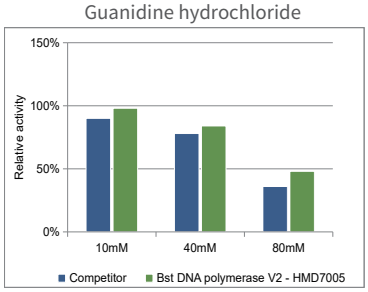
Comparison of enzyme activity with Company N at 30°C and 65°C



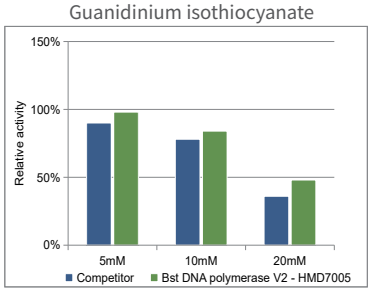
After aptamer modification, WS Bst DNA polymerase V2(HMD7006 and HMD7007) has lower enzymatic activity than Company N at 30 °C and higher blocking efficiency(More beneficial to prevent non-specific amplification). At the working temperature(65°C), the activity is released and is higher than that of Company N. In an RT-LAMP reaction, NTC does not appear within 60 minutes.

Inhibitor tolerance

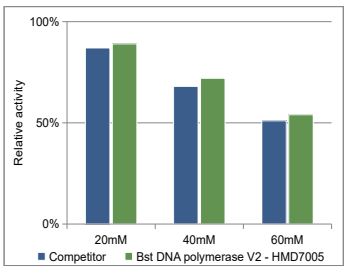
Guanidine hydrochloride



Guanidinium isothiocyanate

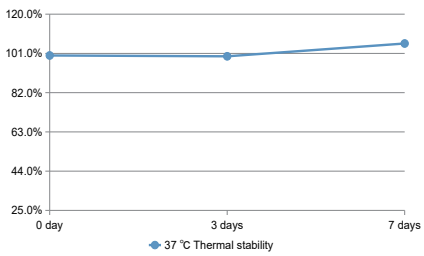


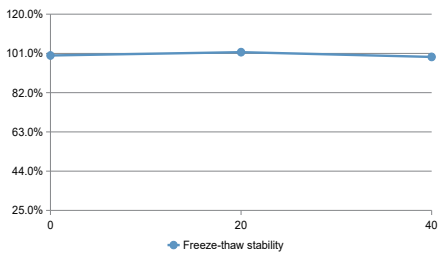
KCL



Hzymes Biotech's Bst DNA polymerase V2(HMD7005) is compared with competing products in different inhibitory environments (guanidine hydrochloride, guanidine isothiocyanate, KCl, etc.), Hzymes Biotech's Bst DNA polymerase V2(HMD7005) is more robust than the competitive products and more suitable for different application scenarios, such as direct amplification. It is easier for the subsequent development of LAMP/RT-LAMP reagent.

Glycerol-free and Stability





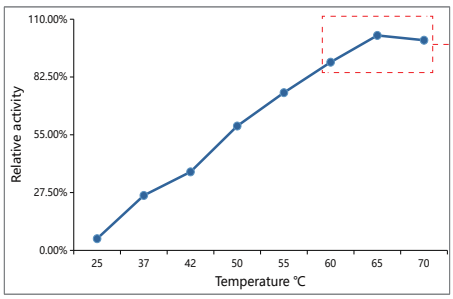
Bst DNA polymerase V2 and WS Bst DNA polymerase V2 products maintain the same activity after incubation at 37°C for 0, 3, and 7 days. To ensure the development of LAMP/RT-LAMP lyophilized reagents, the activity of glycerol-free Bst DNA polymerase V2 series (HMD7005, HMD7006, HMD7007) remains unchanged after repeated freeze-thaw cycles 40 times.

RTL REVERSE TRANSCRIPTASE 2.0

RTL Reverse Transcriptase 2.0 is a brand-new reverse transcriptase from a different source. It can maintain enzyme activity at higher temperatures(RT-LAMP working temperature) and has RNase H activity. It has the advantages of high reverse transcription efficiency, good freeze-thaw, thermal stability, and strong inhibitor tolerance. Compared with other reverse transcriptases, RTL reverse transcriptase 2.0 is more suitable for developing the RT-LAMP reagent. In addition, the RTL Reverse Transcriptase 2.0 series products include glycerol-free and aptamer-modified versions, which are more helpful for developing lyophilized RT-LAMP reagents and preventing false positive results.

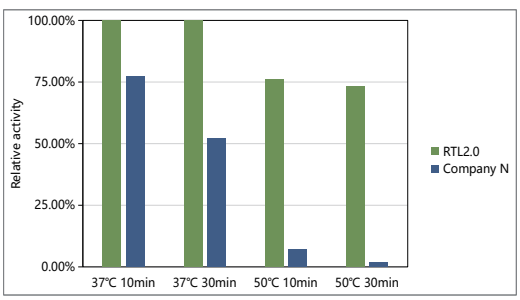
Reverse transcription efficiency

The activity of RTL reverse transcriptase 2.0 at different temperatures



Hzymes Biotech's RTL reverse transcriptase 2.0(HMD5302) has excellent heat resistance, and its optimum activity range perfectly adapts to the reaction conditions of RT-LAMP (60-65 °C). Its reverse transcription efficiency is high, and can be completed within 1-3 minutes.

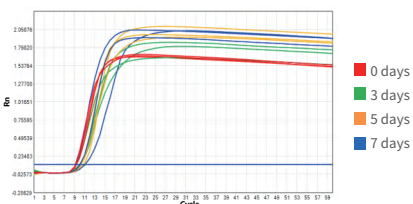
Thermal stability at higher temperature



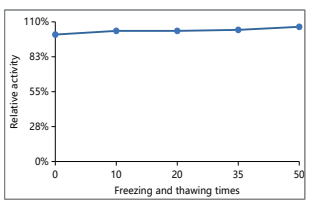
Compared with Company N, Hzymes Biotech's RTL reverse transcriptase 2.0(HMD5302) has higher activity and better stability when incubated at 37 °C and 50 °C for 30 min and 10 min).

Glycerol-free and stability

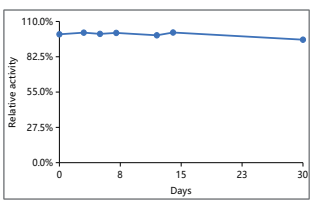
37 °C Thermal stability for 7 days



Freeze-thaw stability at -20 °C



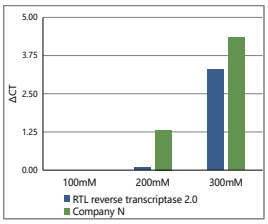
30-days stability at 25°C



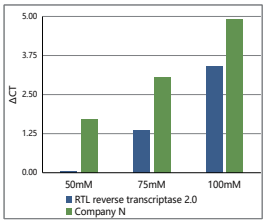
Hzymes Biotech's RTL reverse transcriptase 2.0(HMD5302), incubated at 25°C for 30 days and incubated at 37°C for 7 days, the reverse transcription performance of RT-LAMP did not change, and the activity maintained the same. To ensure the development of RT-LAMP lyophilized reagents, the activity of glycerol-free RTL reverse transcriptase 2.0(HMD5302) remained unchanged after repeated freeze-thaw cycles 50 times.

Inhibitor tolerance

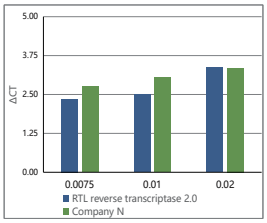
Guanidine hydrochloride



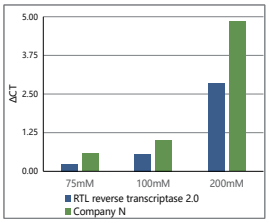
Guanidinium isothiocyanate



Whole blood



NaCl

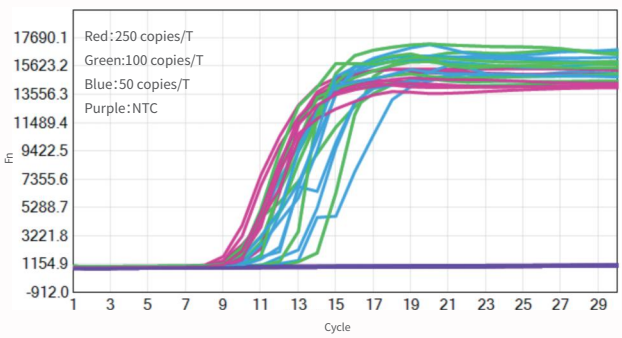


Guanidine hydrochloride and guanidine isothiocyanate are common nucleic acid extraction residues. RTL reverse transcriptase 2.0 (HMD5202) activity is higher than that of Company N under the same inhibition concentration. It is better than competing products with NaCl and whole blood(including single components). It is more suitable for various application scenarios, such as direct amplification, and easier for the subsequent development of RT-LAMP reagent.

RT-LAMP MASTER MIX(FLUORESCENT)
- LIQUID & LYOPHILIZED

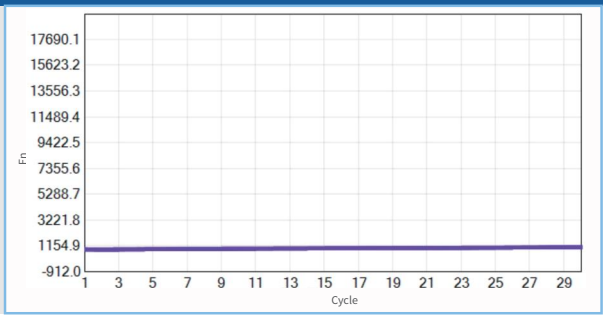
High sensitivity and high inhibitor tolerance

RT-LAMP reaction is performed using a nasal swab sample spiked with SARS-CoV-2 pseudovirus(50 copies/T, 100 copies/T, and 250 copies/T) after a simple treatment with Hzymes Sample release buffer (HMD3504).



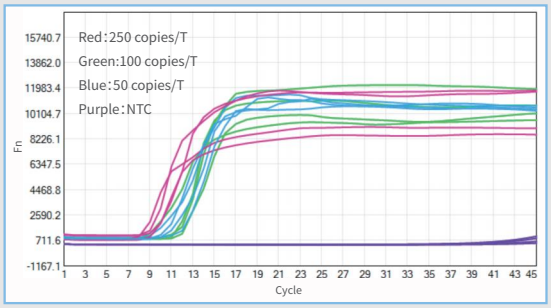
High specificity

There is no NTC amplification while using RT-LAMP fluorescent master mix(Liquid, lyophilization ready) (HMD5207).



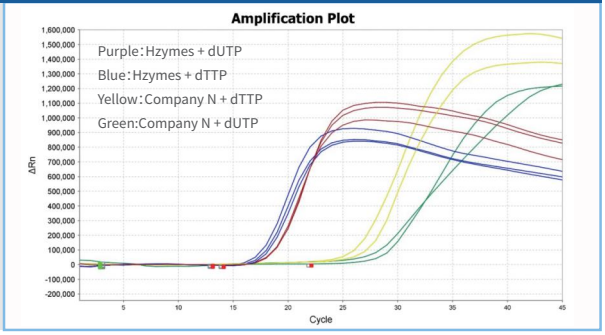
Performance consistency with lyophilized reagent

The data illustrates that the lyophilization process does not affect the performance of the RT-LAMP reaction. (The same template concentration: 250 copies/T, 100 copies/T, and 50 copies/T).

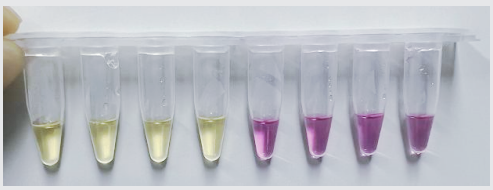


dUTP tolerance

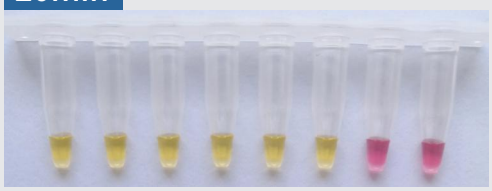
Hzymes RT-LAMP fluorescent master mix(Liquid, lyophilization ready)(HMD5207) performs better dUTP tolerance than company N, while using the same primer set and template.

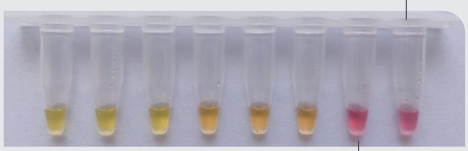


RT-LAMP MASTER MIX(COLORIMETRIC)
- LIQUID & LYOPHILIZED



20min





Good thermal stability after 37 °C for 14 days.

Before the reaction

The nasal swab samples spiked with SARS-CoV-2 pseudovirus(1 cp/μL、2 cp/μL、5 cp/μL)can be 100% detected using RT-LAMP colorimetric master mix (Liquid, lyophilization ready) (HMD5204). The samples have been simply treated using Hzymes Sample release buffer(LAMP colorimetric) (HMD3505).

SELECTION GUIDE
FOR YOUR LAMP APPLICATION

LAMP/RT-LAMP raw materials

Hzymes Cat. No.	Products	Specification
HMD7004	Bst DNA polymerase V2	1600U, 8000U, 80000U
HMD7005	Bst DNA polymerase V2(Glycerol-free)	1600U, 8000U, 80000U
HMD7006	WS Bst DNA polymerase V2(Glycerol-free)	1600U, 8000U, 80000U
HMD7007	WS Bst DNA polymerase V2 (High concentration glycerol-free)	1600U, 8000U, 80000U
HMD5302	RTL reverse transcriptase 2.0(Glycerol-free)	1500U, 15000U,150000U
HMD5303	WS RTL reverse transcriptase 2.0	1500U, 15000U,150000U
HMD5304	WS RTL reverse transcriptase 2.0(Glycerol-free)	1500U, 15000U,150000U
HMD3901A	RNase inhibitor(Glycerol-free)	2000U, 20000U, 200000U
HMD2101	dNTP(25mM each)	0.5mL, 1mL, 5mL, 100mL
HMD2102	dNTP(10mM each)	0.5mL, 1mL, 5mL, 100mL
HMD3504	Sample release buffer	1mL, 8mL, 100mL, 1000mL
HMD3505	Sample release buffer(LAMP colorimetric)	1mL, 8mL, 100mL, 1000mL

LAMP/RT-LAMP related CDMO service

Hzymes Cat. No.	Products
HCRO0011	LAMP primer design and optimization
HCRO0012	The development of lyophilization process
HCRO0013	Contract production of lyophilized reagent

LAMP/RT-LAMP master mix

Hzymes Cat. No.	Products	Specification
HMD5204	RT-LAMP colorimetric master mix (Liquid, lyophilization ready)	96RXN, 960RXN, 9600RXN
HMD5205	RT-LAMP colorimetric master mix (In situ lyophilization)	96RXN, 960RXN, 9600RXN
HMD5206	RT-LAMP colorimetric master mix (Lyophilized beads)	96RXN, 960RXN, 9600RXN
HMD5207	RT-LAMP fluorescent master mix (Liquid, lyophilization ready)	96RXN, 960RXN, 9600RXN
HMD5208	RT-LAMP fluorescent master mix (In situ lyophilization)	96RXN, 960RXN, 9600RXN
HMD5209	RT-LAMP fluorescent master mix (Lyophilized beads)	96RXN, 960RXN, 9600RXN
HMD5310	RT-LAMP fluorescent probe master mix (Liquid, lyophilization ready)	96RXN, 960RXN, 9600RXN
HMD5311	RT-LAMP fluorescent probe master mix (In situ lyophilization)	96RXN, 960RXN, 9600RXN
HMD5312	RT-LAMP fluorescent probe master mix (Lyophilized beads)	96RXN, 960RXN, 9600RXN

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