

RT-LAMP Control Reaction

This reaction can be used if ever in doubt that your RT-LAMP is not working correctly. This reaction allows you to ensure the reverse transcriptase, LAMP mastermix and Genie® instrument are all working.

Primer Details

Primer Name	Primer Sequence
RNA_Control_F3	TTACAAACCAGCATCCGTAG
RNA_Control_B3	CATATGACTCGTTATAGCGGAC
RNA_Control_FIP	CATAGGAGCACCGTTGGAGAACCTTATTGGCAACCTCCTCTC
RNA_Control_BIP	ATGCAGCGCCTTACAAGAAGTTCCTCGGACCAATAGAGCC
RNA_Control_LF	ACAACGACGATCGGTAGC
RNA_Control_LB	CTGAACAAGCAACCGTTACC

TTACAAACCAGCATCCGTAGCCTTATTGGCAACCTCCTCTCTGGCTACCGATCGTCGTTGTTGGGCAATGCACG
 TTCTCCAACGGTGCTCCTATGGGGCACAAGTTGCAGGATGCAGCGCCTTACAAGAAGTTCCTGAACAAGCAACC
 GTTACC CCCC GCCTCTGAGAGCGGCTCTATTGGTCCGAGACCAATGTGCGCCGTGGATCAGACACGCGTCCGC
 TATAACGAGTCATATG

Primer Master-Mix

The following primer master-mix should be made. This makes enough 10x primer mix for 100x 25µl LAMP reactions

Primer	Volume	Final conc. in 25µl LAMP
RNA_Control_F3	5µl	0.2µM
RNA_Control_B3	5µl	0.2µM
RNA_Control_FIP	20µl	0.8µM
RNA_Control_BIP	20µl	0.8µM
RNA_Control_LF	10µl	0.4µM
RNA_Control_LB	10µl	0.4µM
10mM Tris-HCL (pH8.0)*	180µl	N/A

*alternatively nuclease free water can be used

Template

The target template for this assay is MS2 RNA, we use MS2 RNA bought in from Sigma-Aldrich (now Merck).

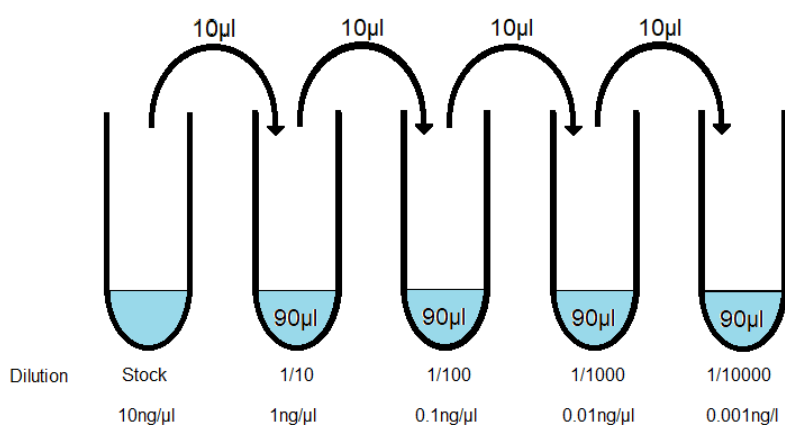
If preferred this template and primer mix are available for purchase using catalogue number is: **CR-MS2-050**.

RT-LAMP Control Reaction

Template Dilution

Make a 1:10 serial dilution of a 10ng/μl stock of MS2 RNA to get a working stock of 0.001ng/μl

1. Dispense 90μl nuclease free water into 4 tubes
2. Transfer 10μl of the 10ng/μl MS2 stock into the first dilution tube containing the pre-dispensed 90μl water and mix thoroughly. This creates a concentration of 1ng/μl MS2
3. Transfer 10μl of the 1ng/μl MS2 second dilution tube containing the pre-dispensed 90μl water and mix thoroughly. This creates a concentration of 0.1ng/μl
4. Repeat this pattern to complete the full dilution series to reach a final concentration of 0.001ng/μl



Control RT-LAMP Reaction

A control reaction should contain the following

	1x 25μl
LAMP Master-mix (ISO-001 or ISO-004)	15μl
10x primer mix	2.5μl
AMV (1U/μl)	0.5μl
Ms2 RNA (0.001ng/μl)	5.0μl
Water	3.5μl

Master-Mix	Isothermal Reaction	Anneal Step
ISO-001	30mins @ 65°C	98-70°C @ 0.05°C/sec
ISO-004	20mins @ 65°C	98-70°C @ 0.05°C/sec

Expected anneal temperature for this reaction is 88.4°C +/- 1°C

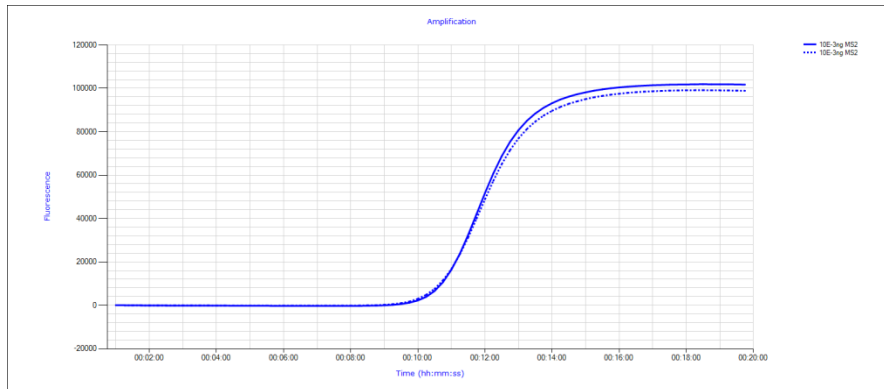
RT-LAMP Control Reaction

Expected Results

These graphs show the above reaction being run using ISO-004. Using ISO-001 would see slower reaction times than those seen here

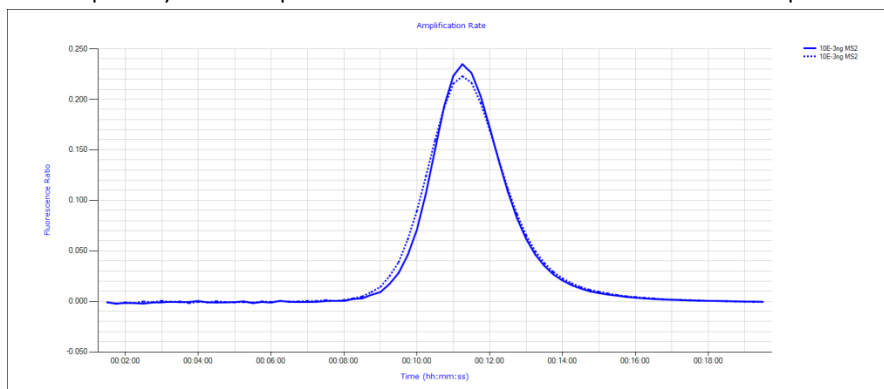
Isothermal Amplification

Detection of the target well within the 20min time frame, fluorescence quickly increases to give a steep amplification curve that quickly reaches a plateau phase



Amplification rate

Peak detection time of 11:15minutes using ISO-004. The amplification reaches peak time quickly with a peak that is even and narrow in shape



Anneal Derivative

Anneal curve shows a sharp increase to a defined and narrow peak. The peak is even in shape and has no additional bumps, peaks for shoulders. T_a of the peak is $88.4^{\circ}\text{C} \pm 1^{\circ}\text{C}$

