Unit 5 Blatchford Road ♦ Horsham ♦ West Sussex ♦ RH13 5QR ♦ UNITED KINGDOM
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Genie® Control Reaction

This document provides you with the details of making a reliable control assay that can be used to reassure you that the Genie® is working correctly, if ever in doubt, and can also be used as a generic positive control to test for inhibitors in samples.

If preferred, the control reaction primer mix and template pre-prepared can be purchased using catalogue number: **CD-ECO-050**

Primers and a synthetic DNA of the target should be ordered for testing

Primer Details

Primer name	Primer Sequence
DNA_Control_F3	CGGTATTGCCGAAGTTCT
DNA_Control_B3	GCATTAACTCAGCCAGCA
DNA_Control_FIP	AAATCGTCGCACCCAGATTCATATCAGATCAGTGGTTCCGA
DNA_Control_BIP	TACGTGATGCCAGCGTGGGCGACAATTTCCGGGTTA
DNA_Control_LF	TTAACTGCTGCGTGACCG
DNA_Control_LB	GTTGTTTCCAGCGCGATTT

Primer Master-Mix

Primers should be made to a concentration of 100µM using 10mM Tris-HCl (pH8.0) or nuclease free water

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
DNA_Control_F3	5µl	0.2µM
DNA_Control_B3	5µl	0.2µM
DNA_Control_FIP	20µl	0.8µM
DNA_Control_BIP	20µl	0.8µM
DNA_Control_LF	10µl	0.4µM
DNA_Control_LB	10µl	0.4µM
10mM Tris-HCl (pH8.0)*	180µl	N/A

*alternatively nuclease free water can be used

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Target DNA Sequence

Cggtattgccgaagttctggccaatgaaggttatcagatcagtggttccgatttagcgccaa
atccggtcacgcagcagttaatgaatctgggtgcgacgatttatttcaaccatcgcccggaa
aacgtacgtgatgccagcgtggtcgttgtttccagcgcgatttctgccgataacccggaaat
tgtcgccgctcatgaagcgcgtattccggtgatccgtcgtgccgaaatgctggctgagttaa
tgc

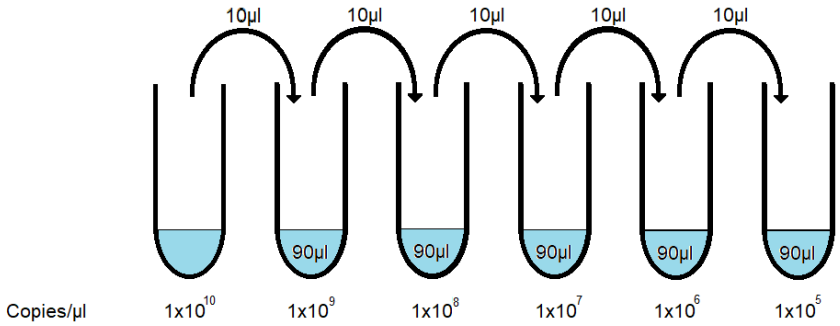
We order our synthetic DNA as gBlocks from Integrated DNA Technologies (IDT). They arrive as lyophilised 500ng pellets. For this particular gBlock, at 251bp, one 500ng pellet contains $1.85x10^{12}$ copies of the synthetic DNA target. Adding 185µl of nuclease free water gives a concentrated stock of synthetic DNA at $1x10^{10}$ copies/µl.

The gBlock can be used at any concentration above $1x10^5$ copies/µl as a control in a LAMP reaction. For our example we have diluted it to a working stock of $1x10^5$ copies/µl

Template gBlock Dilution

Make a 1:10 serial dilution of your concentrated stock of gBlock to give you a suitable working concentration to test. We recommend a working stock of $1x10^5$copies/µl to be made

1. Add 185µl nuclease free water to the 500ng lyophilised 251bpgBlock. This makes a $1x10^{10}$ copies/µl stock
2. Dispense 90µl nuclease free water into 5 tubes
3. Transfer 10µl of the $1x10^{10}$ copies/µl gBlock stock into the second dilution tube containing the pre-dispensed 90µl water and mix thoroughly. This creates a concentration of $1x10^9$copies/µl
4. Transfer 10µl of the $1x10^9$ copies/µl gBlock into the first dilution tube containing the pre-dispensed 90µl water and mix thoroughly. This creates a concentration of $1x10^8$copies/µl
5. Repeat this pattern to complete the full dilution series to reach a final concentration of $1x10^5$ copies/µl



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Control LAMP Reaction

A control reaction should contain the following

	1x 25µl
LAMP Master-mix (ISO-001 or ISO-004)* 10x primer mix 1x10^{5}copies/µl gBlock	15µl 2.5µl 5µl
Water	2.5µl

*if an LNL master-mix is being used then please ensure a final concentration of 60mM KOH is added to the reaction otherwise the LAMP reaction will fail

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

This control reaction should have an anneal temperature of 88°C +/- 1°C. Please note the Tm will vary if using LNL master mix or if altering the ramping speed from the default 0.05°C/s on the anneal step

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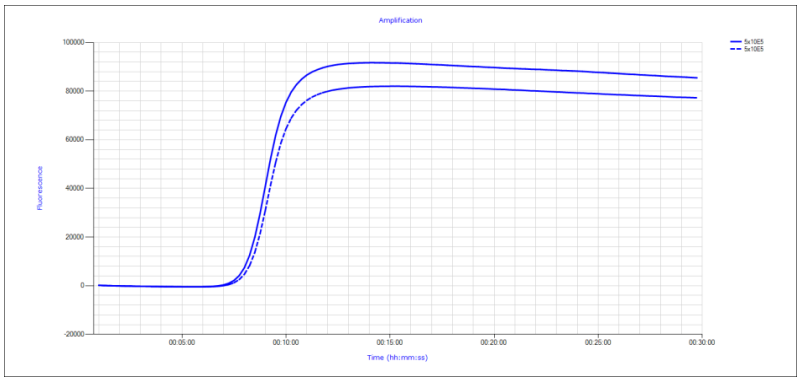
Expected Results

These graphs show the above reaction being run using ISO-001. Using ISO-004 should be a faster reaction than those reported here.

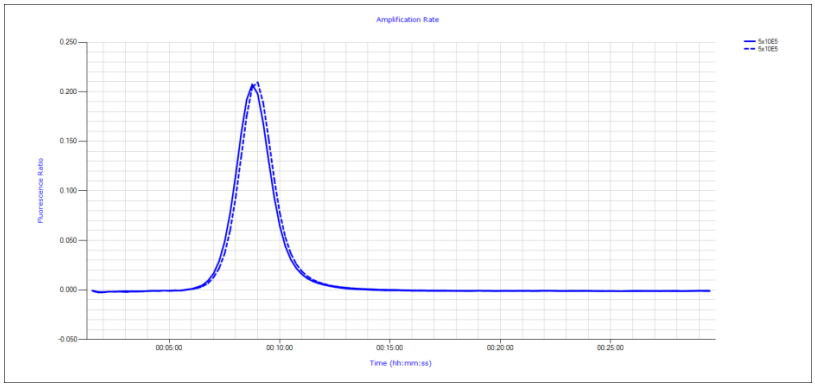
Isothermal Amplification

Early detection of the target and fluorescence quickly increases giving a steep amplification curve. This then quickly reaches a plateau phase.



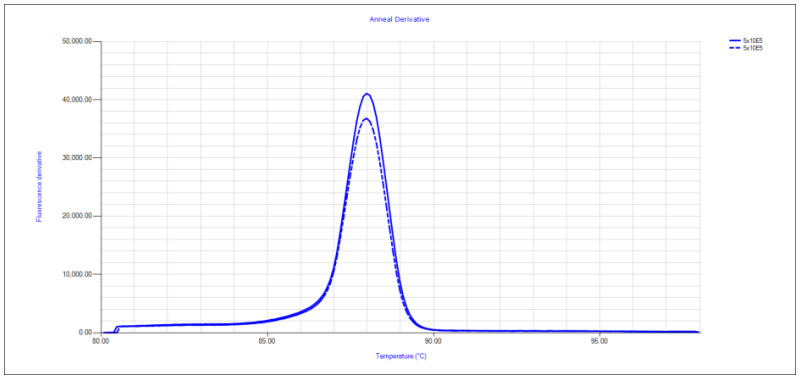
Amplification rate

Peak detection time at 08:45minutes using ISO-001. The amplification rate reaches peak time very quickly with the peak 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 peaks, bumps or shoulders. Please note that using an LNL master-mix or altering the ramp rate of the anneal step from the default 0.05°C/s will shift the anneal temperature seen.



Expected anneal temperature using ISO-001 with an anneal ramp rate of 0.05°C/s is 88°C +/-1°C

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Testing for Inhibitors in a sample

These control DNA and primers can also be used to assess the level of inhibitors in a sample. By comparing amplification of the control reaction by itself against the same reaction containing the sample of interest the presence of inhibitors can be determined

Reaction setup for standard master mixes

	Control	Sample to be tested
ISO-001 or ISO-004 10x control primer mix 1x10^{5}copies/µl gBlock Sample DNA	15µl 2.5µl 2.5µl -------	15µl 2.5µl 2.5µl 1-5µl*
Water	5µl	To 25µl

*this should be the sample size you intend to use in your target LAMP assays

Reaction setup for LNL master mixes

	Control	Sample to be tested
ISO-001LNL or ISO-004LNL 10x control primer mix 1x10^{5}copies/µl gBlock Sample DNA (in 0.3M KOH) 0.3M KOH	15µl 2.5µl 2.5µl ------- 5µl	15µl 2.5µl 2.5µl 5µl ------
Water	0µl	0µl

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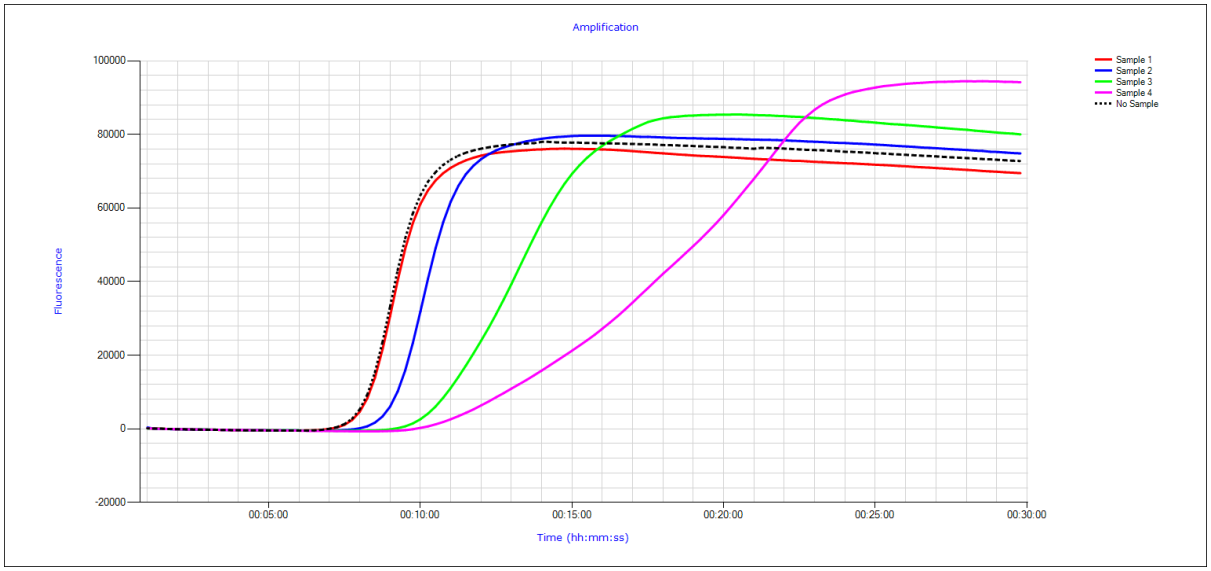
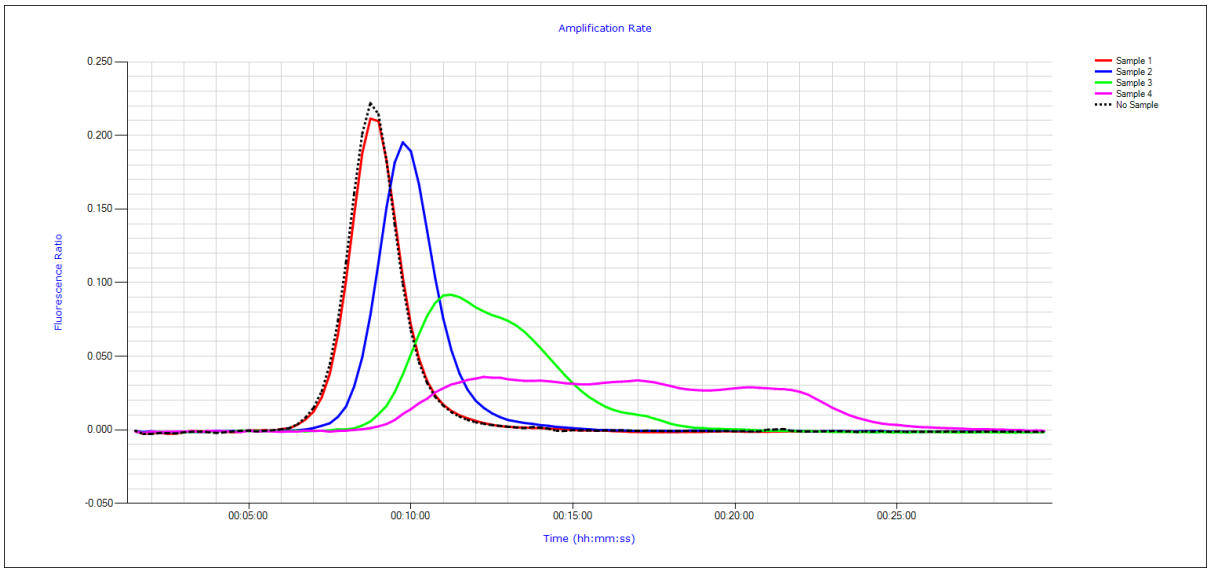
Results of an example sample inhibitor test

Amplification



Amplification Rate



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Analysis of Results

Sample	Amplification	Amplification Rate	Conclusion
Control (no sample)	Early detection, sharp increase in fluorescence, quickly reaches plateau	High, sharp, narrow even peak. Early peak detection	What you would expect to see in a sample with no inhibitors
Sample 1	Same as control	Same as control	No inhibitors present
Sample 2	Looks the same as for control just with a delay before the start of the detection	Looks the same as for control just with a delay before the peak detection. Slightly lower peak height	Slight inhibitor present delaying reaction but should be perfectly useable even on a slower assay or a lower target DNA concentration
Sample 3	Fluorescence detection beings later, shallower gradient	No longer a clean defined peak. Peak is now lower, broader and slightly uneven	Higher amount of inhibitor. Likely useable but could cause an issue with slower assays or lower DNA concentrations
Sample 4	Late detection of fluorescence, gradient now very shallow and becomes uneven	Not recognisable as having a defined peak. It is very broad, low and uneven	High level of inhibitor. Will likely cause false negatives especially with slow assays or low DNA concentrations
Sample 5	No amplification	No amplification	Completely inhibitory, unusable