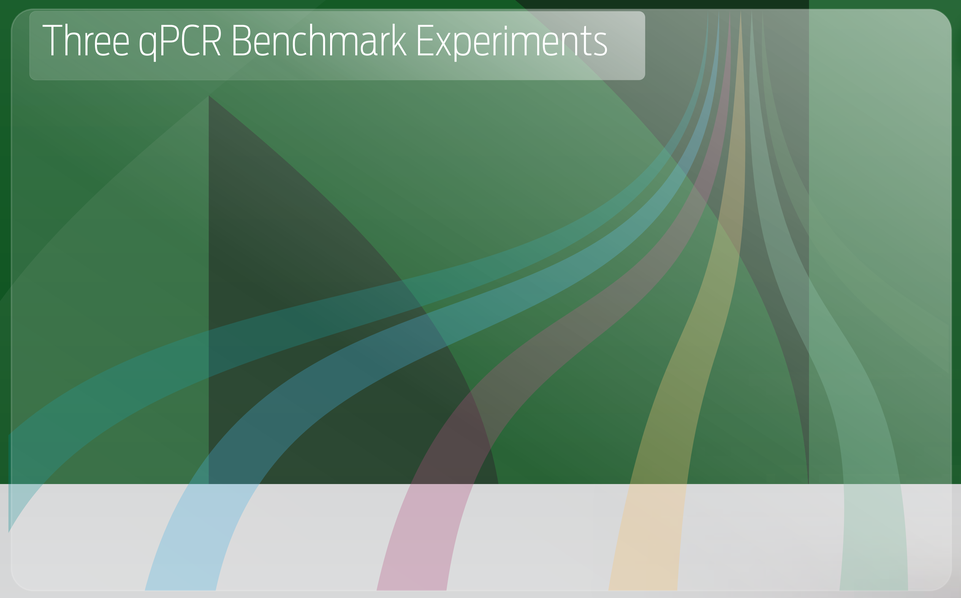
Benchmark Analysis

NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix

Product No MB442



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Enhanced Detection and Robust Amplification

NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix is a high performing master mix, combining sensitivity and efficiency for fast and accurate results.

Benchmarked against a total of 4 market-leading master mixes considered to be the gold-standard in qPCR Master Mixes, the **MB442** proved to be a leading-edge product with first-class results.

Amplification of **SARS-CoV-2** genes in different contexts

A 10-fold serial dilution of RNA carrying the SARS-CoV-2 genes RdRp (for virus RNA dependent RNA polymerase) and N (for Nucleocapsid phosphoprotein) was used as template to detect SARS-CoV-2 in three different real-time qPCR experiments:

I. The reaction mixture contains primers and probes to amplify SARS-CoV-2 genes (RdRp and N), in the presence of SARS-CoV-2 RNA.

II. The reaction mixture contains, in addition to SARS-CoV-2 primers and probes to amplify RdRp and N genes, primers and probes to simultaneously detect Influenza A (INFA) and Influenza B (INFB), as well as human RNase P, although only SARS-CoV-2 RNA is present.

III. The reaction mixture contains, in addition to SARS-CoV-2 primers and probes to amplify RdRp and N genes, primers and probes to simultaneously detect Influenza A (INFA) and Influenza B (INFB), as well as human RNase P, in the context of a co-infection scenario where nucleic acids of all different species are present.

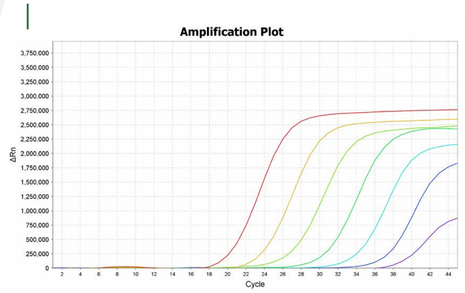
Probes were differently labeled to allow simultaneously detection of the three species –SARS-CoV-2 (JUN); Influenzavirus A and B (FAM); human RNase P (VIC).

Comparison of Ct variation in different contexts to detect SARS-CoV-2 RNA

When comparing **NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix** with a similar mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of speed.

NZYTech | #MB442

I



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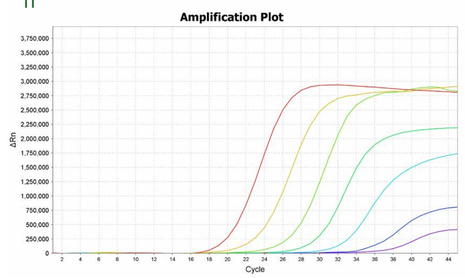

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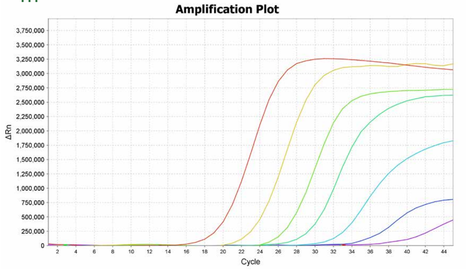
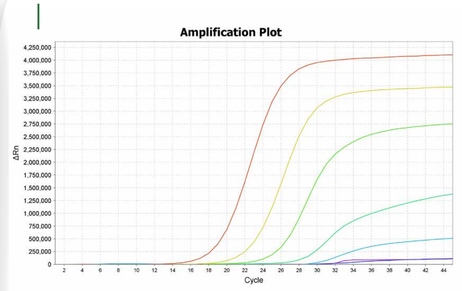


Competitor AB

I



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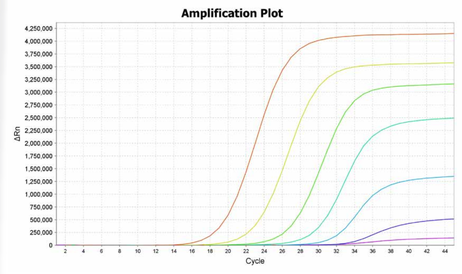

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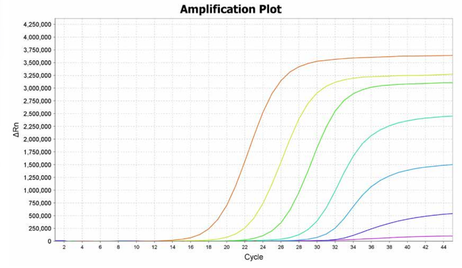
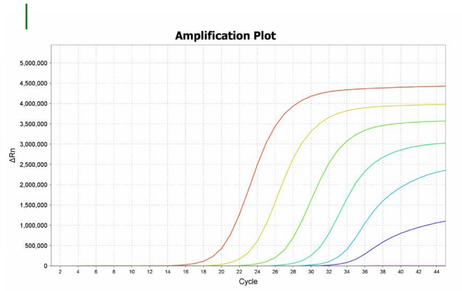


Competitor B

I



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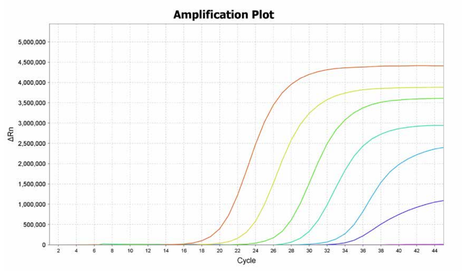

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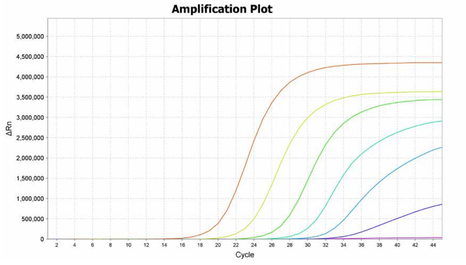
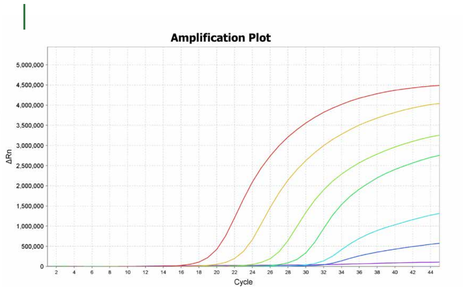


Competitor P

I



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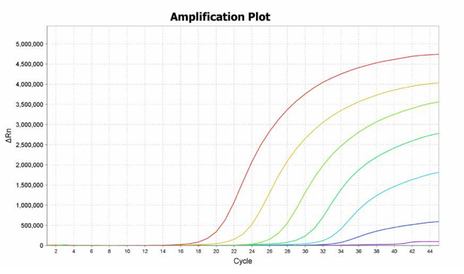

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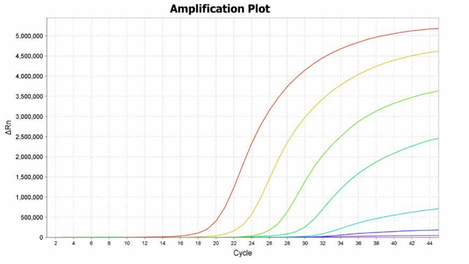
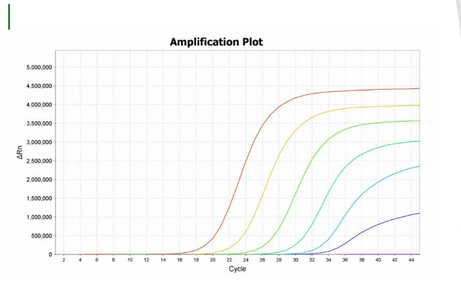


Competitor MB

I



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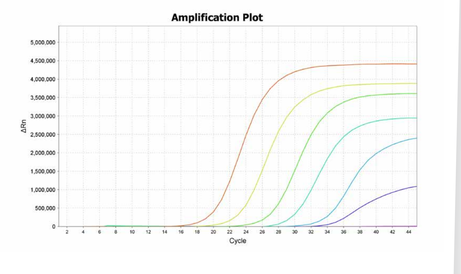

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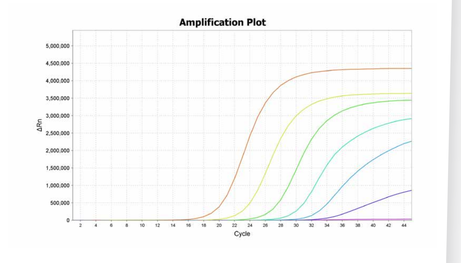


Note: In condition III, amplification curves corresponding to detection of INFA (FAM), INFB (FAM) and RNase P (VIC) were not included in the plots. JUN channel used for RdRp and N gene amplification

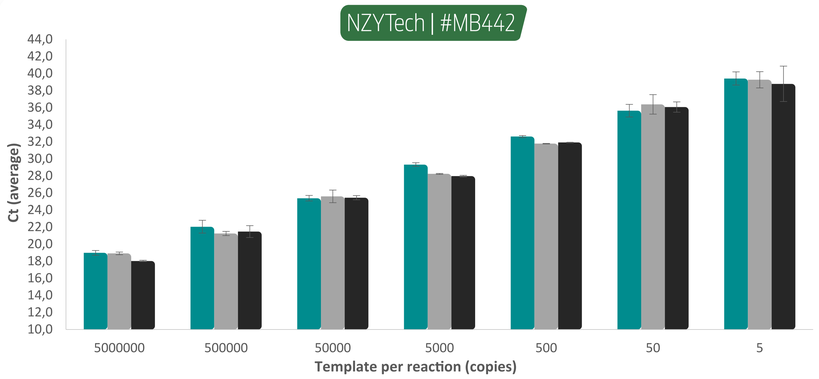
I: The reaction mixture contains primers and probes to amplify SARS-CoV-2 genes (RdRp and N), in the presence of SARS-CoV-2 RNA.

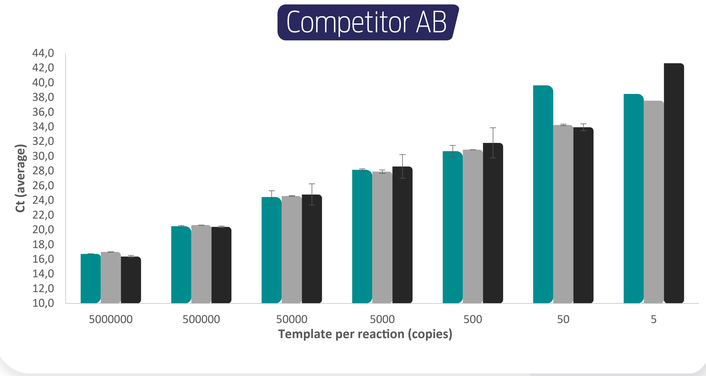
II: The reaction mixture contains, in addition to SARS-CoV-2 primers and probes to amplify RdRp and N genes, primers and probes to simultaneously detect Influenza A (INFA) and Influenza B (INFB), as well as human RNase P, although only SARS-CoV-2 RNA is present.

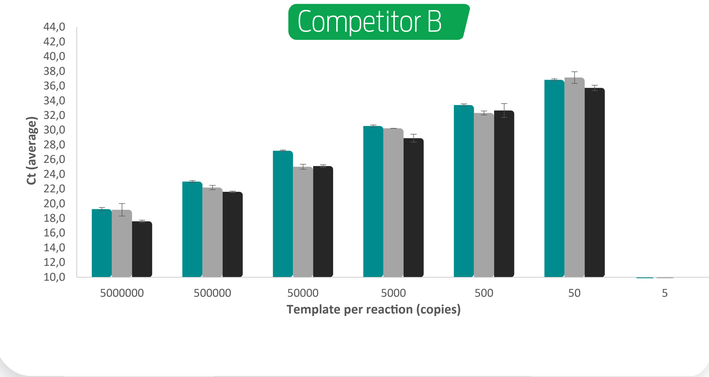
III: The reaction mixture contains, in addition to SARS-CoV-2 primers and probes to amplify RdRp and N genes, primers and probes to simultaneously detect Influenza A (INFA) and Influenza B (INFB), as well as human RNase P, in the context of a co-infection scenario where nucleic acids of all different species are present.

Comparison of Ct variation in different contexts to detect SARS-CoV-2 RNA

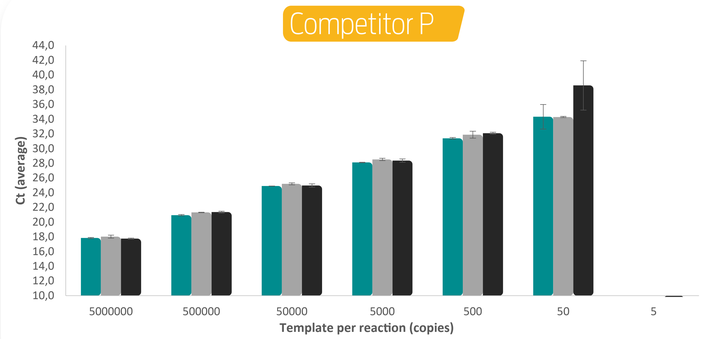
NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix shows high-performance when comparing Ct variation in all three qPCR experiments. The superior sensitivity of NZYTech's master mix resulted in reproducible amplification of up to 5 copies of template in all contexts to detect SARS-CoV-2 rRNA, by contrasting with market-leading master mixes. Multiplexing does not affect sensitivity of NZY-Supreme Multiplex One-Step RT-qPCR Probe Master Mix.

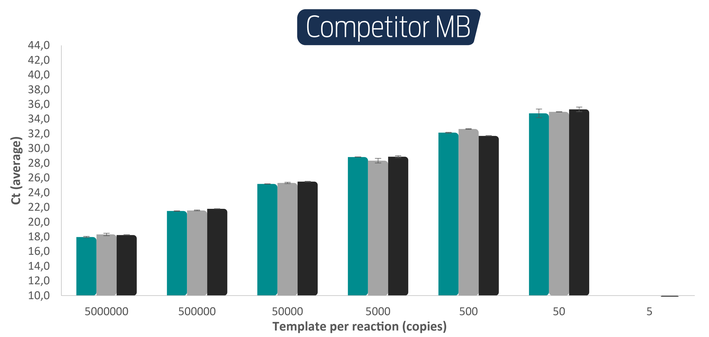






- I) Detection of SARS-CoV-2 RNA (primers for RdRp and N)
- II) Detection of SARS-CoV-2 RNA (primers for RdRp, N, INFA, INFB and hRP)
- III) Detection of SARS-CoV-2 RNA in a human sample containing RP with co-infection with INFA & INFB (primers for RdRp, N, INFA, INFB and hRP)





Leading-edge master mix

Optimized and highly efficient reaction mixture developed for simultaneously first-strand cDNA synthesis and subsequent real-time PCR of two or more targets in a single tube.

This master mix was engineered with a dual hot-start enzyme control mechanism to provide the highest detection sensitivity.

DUAL-HOT-START MODE

qPCR

Probe Detection Technology

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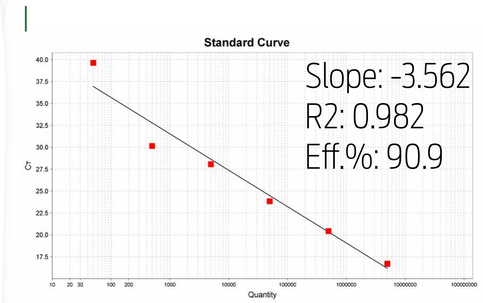
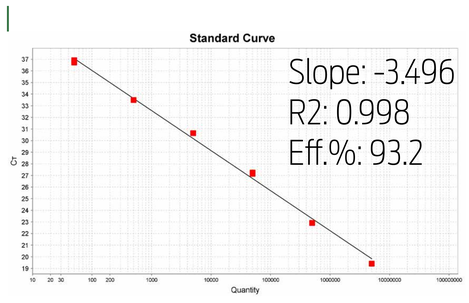
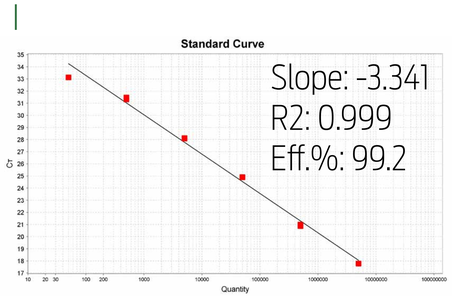
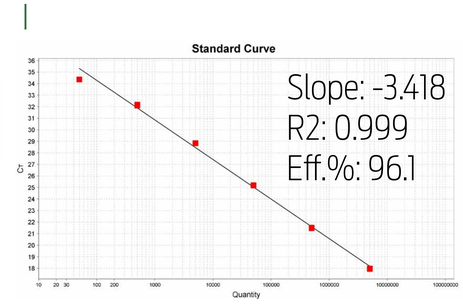
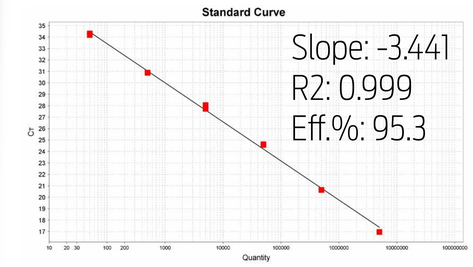
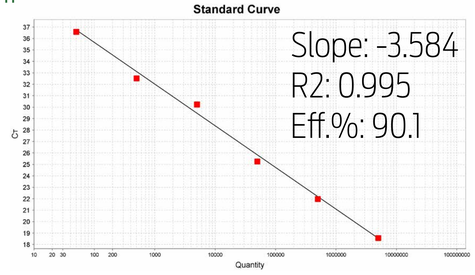
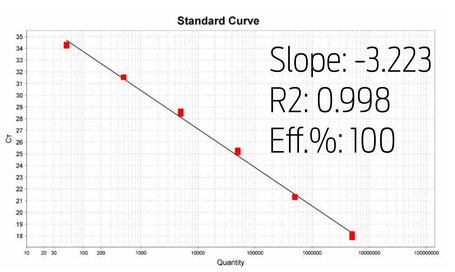
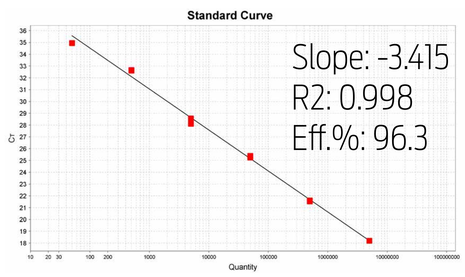
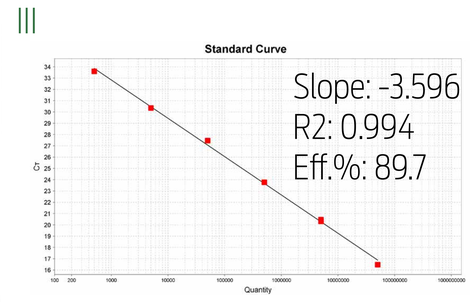
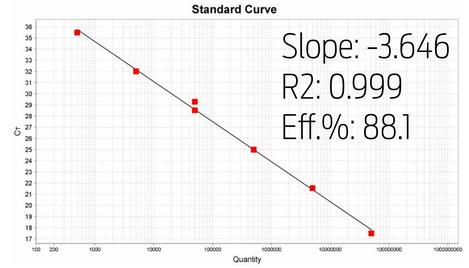
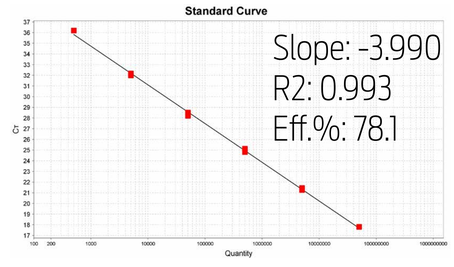
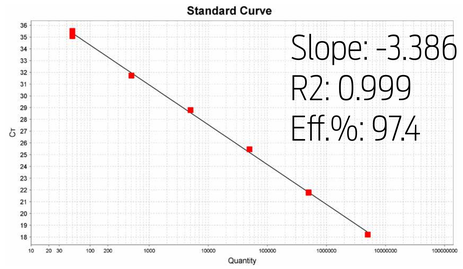
genes & enzymes

Comparison of efficiency in different contexts to detect SARS-CoV-2 RNA

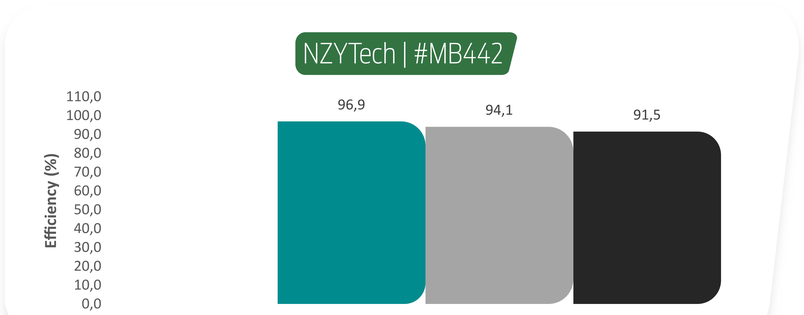
NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix provides high efficiency and linear range over a high number of simultaneous amplifications. The results for SARS-CoV-2 detection in different contexts show excellent PCR efficiency and R2 values even when the NZYTech's master mix is used for the simultaneously detection of 5 different targets in a co-infection scenario (assay III).

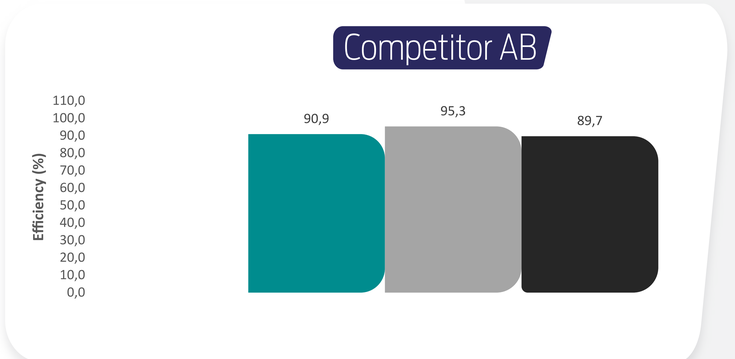
NZYTech | #MB442	Competitor AB	Competitor B	Competitor P	Competitor MB
I				
II				
III				

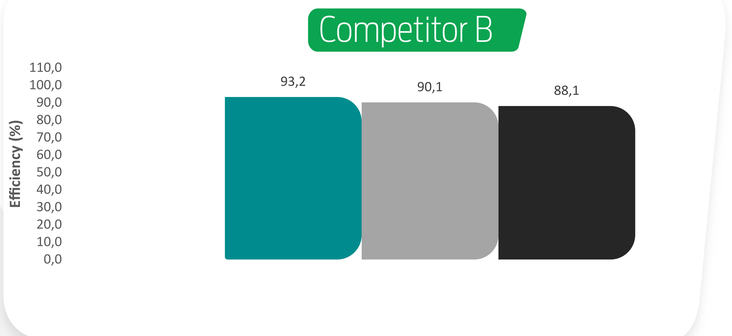
Comparison of efficiency in different contexts to detect SARS-CoV-2 RNA

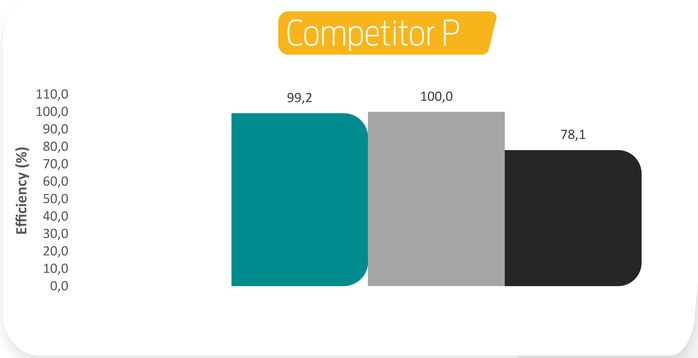
NZYTech's master mix was designed to provide high PCR efficiencies in multiplexing experiments.

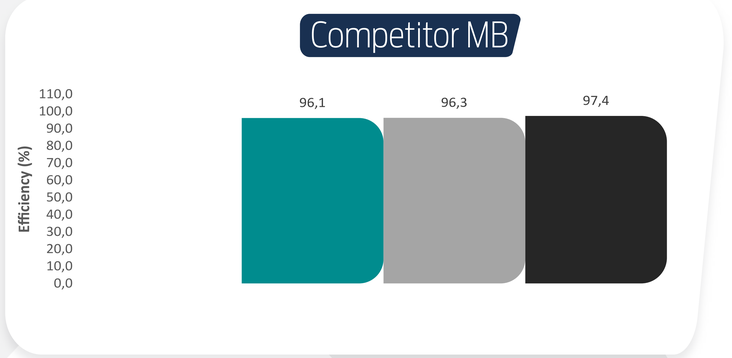
Comparing with market-leading master mixes, efficiency with our master mix is less affected by the number of targets amplified at the same time; when comparing assay I – duplex amplification – with assays II or III – pentaplex amplifications, PCR efficiency remains above 90% when using NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix.











- I) Detection of SARS-CoV-2 RNA (primers for RdRp and N)
- II) Detection of SARS-CoV-2 RNA (primers for RdRp, N, INFA, INFB and hRP)
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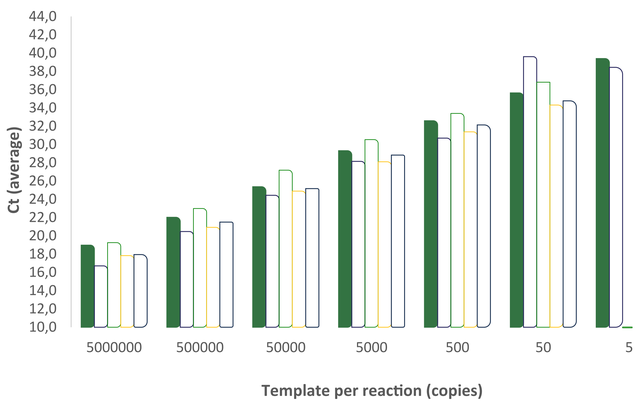
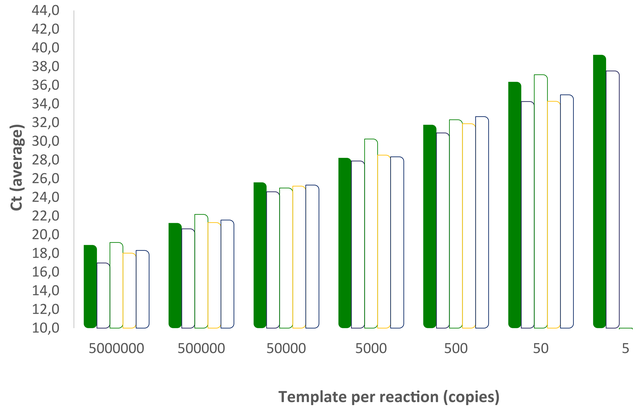
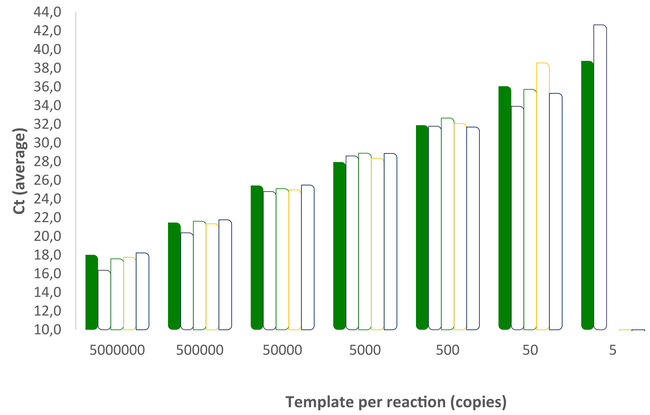
Amplification of SARS-CoV-2 genes in different contexts



Starting Template - SARS-CoV-2 RNA (copies)	$5x10^6$	$5x10^5$	$5x10^4$	$5x10^3$	$5x10^2$	50	5
NZYTech (MB442)	19,0	22,0	25,4	29,3	32,6	35,6	39,4
Competitor AB	16,7	20,5	24,4	28,1	30,7	39,6	38,4
Competitor B	19,3	23,0	27,2	30,5	33,4	36,8	Undetermined
Competitor P	17,8	20,9	24,9	28,1	31,4	34,3	Undetermined
Competitor MB	18,0	21,5	25,2	28,8	32,1	34,8	Undetermined



Starting Template - SARS-CoV-2 RNA (copies)	$5x10^6$	$5x10^5$	$5x10^4$	$5x10^3$	$5x10^2$	50	5
NZYTech (MB442)	18,9	21,3	25,6	28,2	31,8	36,4	39,3
Competitor AB	17,0	20,6	24,6	27,9	30,9	34,3	37,5
Competitor B	19,2	22,2	25,0	30,2	32,3	37,1	Undetermined
Competitor P	18,0	21,3	25,2	28,5	31,9	34,3	Undetermined
Competitor MB	18,3	21,6	25,3	28,3	32,6	35,0	Undetermined



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NZYTech (MB442)	18,0	21,5	25,4	28,0	31,9	36,1	38,8
Competitor AB	16,4	20,4	24,8	28,6	31,8	33,9	42,6
Competitor B	17,6	21,6	25,1	28,9	32,7	35,7	Undetermined
Competitor P	17,8	21,4	25,0	28,4	32,1	38,6	Undetermined
Competitor MB	18,2	21,8	25,5	28,9	31,7	35,3	Undetermined

NZYTech's NZYSupreme Multiplex One-Step RT-qPCR Probe Master mix offers:

Optimal Multiplexing (validated for up to 5 targets)

Superior sensitivity in duplex or even pentaplex gene detection assays, allowing detection of up to 5 copies of RNA

High level signal to detect until to 5 copies of RNA

The best compromise between sensitivity and efficiency whether in duplex or pentaplex assays

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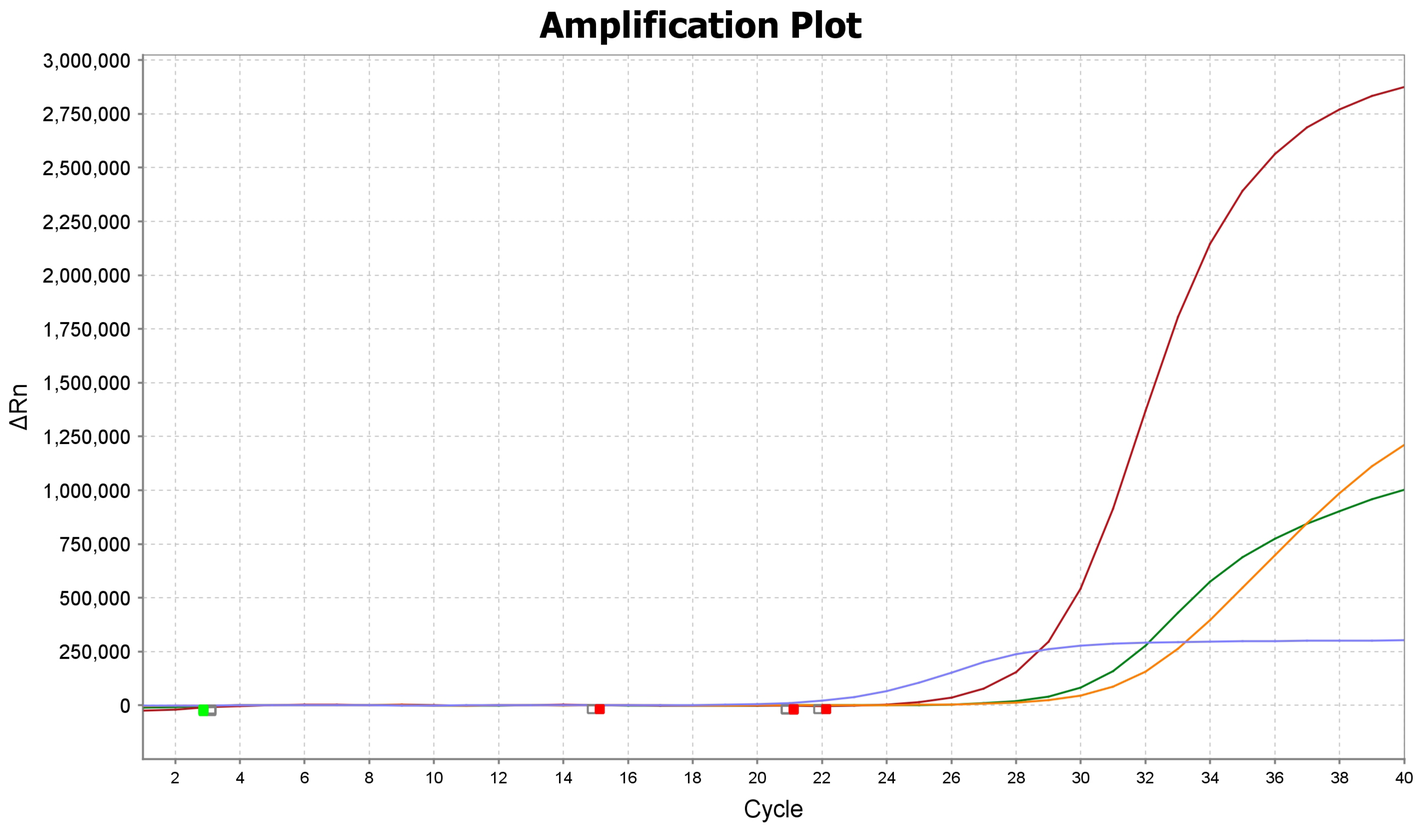
Example of Multiplex Result



Simultaneous detection of five SARS-CoV-2 targets plus one human RNAseP (RP) target from a positive nasopharyngeal sample.

- Red Curve: Detection of the SARS-CoV-2 vRNA target (two targets for N gene) through the FAM channel;
- Orange Curve: Detection of the SARS-CoV-2 vRNA target (two targets for RdRp gene) through the HEX channel;
- Green Curve: Detection of the SARS-CoV-2 vRNA target (one target for E Gene) through the Texas Red channel;
- Purple Curve: Detection of the RP Gene through Cy5 channel

Sensitivity Meets Efficiency

nzytech
molecular biology

NZYSupreme Multiplex One-Step RT-qPCR Probe Master Mix

*For **Enhanced Detection** and **Robust Amplification***

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