

# A Study of the Performance of Desalted Primers Compared to Cartridge and HPLC Purification

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## Overview:

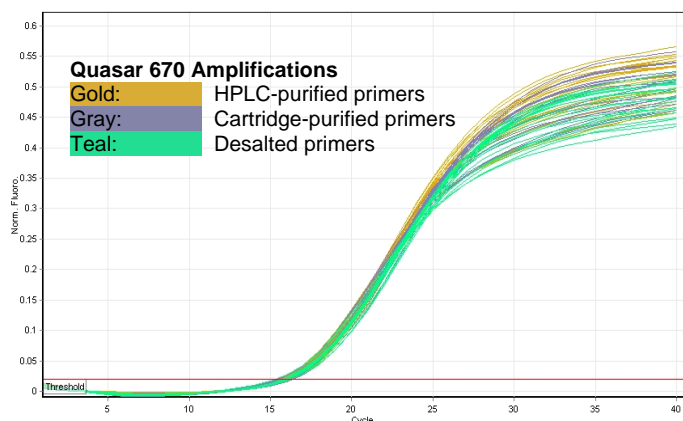
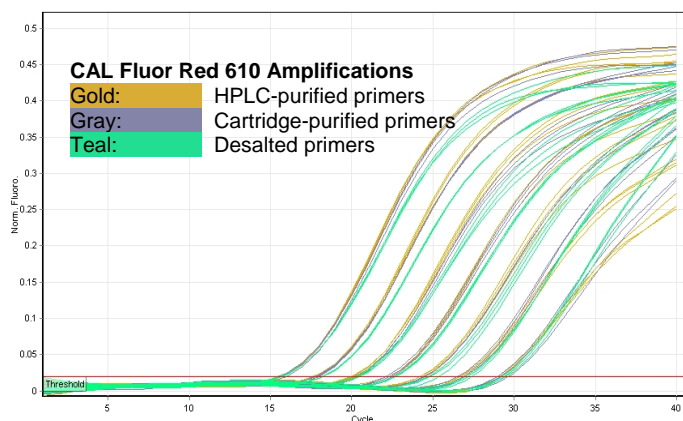
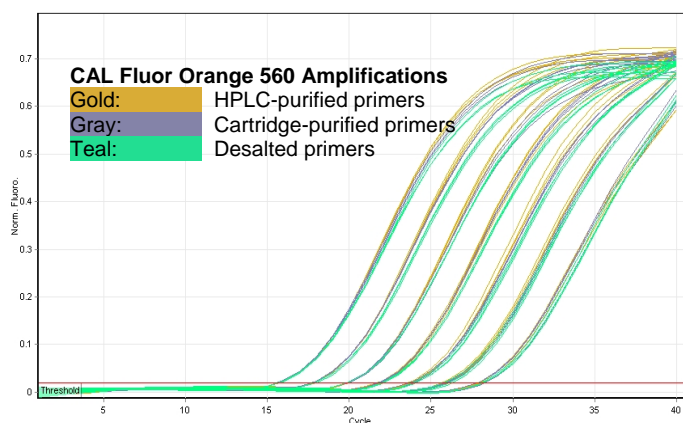
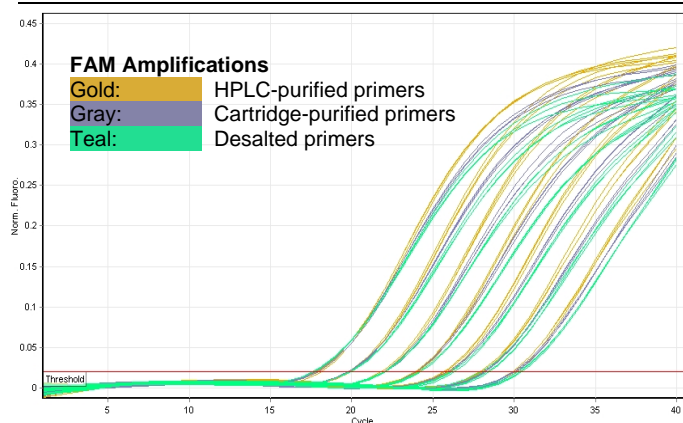
Joseph Knecht, Director of Oligonucleotide Manufacturing recognizes the market demand for desalted primers, particularly among end-users who are price sensitive and are less concerned with the purity specification of their primers. While our current methodology purifies all primers using Reversed Phase Cartridge (RPC), other oligo manufacturers make available desalted primers without any purification as an option to their customers. Before offering the same, we wish to understand any impact from the absence of primer purification upon a 5' nuclease assay. This is tested in the following procedure, which also compares the performance of primers purified more stringently through Reversed Phase HPLC (HPLC).

A quadruplexed 5' nuclease assay was designed for Anthrax detection and presented at the qPCR conference in Germany in 2007. Using synthetic templates instead of sample nucleic acid obtained from the organism, this assay has been adapted into a stringent routine for testing new material formulations for qPCR. One target is present at high concentration (1M copies per reaction), while the other three are each diluted in series down to ~ 250 copies. At the lowest dilution point there is a 4000X difference in the target copies amplified in unison, to rigorously screen for problems with any one oligo component. Each data point is represented by triplicate reactions.

## Reaction Composition:

Reaction Components:	Volume	Concentration:	Assay Sequences:
• Nuclease-free water	0.40 µL	N/A	Pan-Cereus – Chromosomal – Hemolysin III
• 2X MultiMix	10.0 µL	1X	Forward Primer: GGATGGCTCATAATCGTTG
• DNA template (variable)	4.00 µL	variable	Reverse Primer: TTCCACCTGCTAAAAGTAG
• Assay 1 Forward (10 µM)	0.60 µL	300 nM	Probe: [FAM]-TTAAACCACTTTATGAAAATC-[BHQ <sub>plus</sub> ]
• Assay 1 Reverse (10 µM)	0.60 µL	300 nM	
• Assay 1 Probe (10 µM)	0.20 µL	100 nM	<i>anthracis</i> -Specific – Chromosomal – Bacitracin Synthetase
• Assay 2 Forward (10 µM)	0.60 µL	300 nM	Forward Primer: GTCGTATTGCCTAATCTAGTATC
• Assay 2 Reverse (10 µM)	0.60 µL	300 nM	Reverse Primer: GTGAACAGACCGAACATA
• Assay 2 Probe (10 µM)	0.20 µL	100 nM	Probe : [CAL Fluor Orange 560]-TCAGACCTCCCGAAGTAGAAGGA-[BHQ-1]
• Assay 3 Forward (10 µM)	0.60 µL	300 nM	
• Assay 3 Reverse (10 µM)	0.60 µL	300 nM	<i>anthracis</i> -Specific – pXO1 – Lethal Factor Precursor
• Assay 3 Probe (10 µM)	0.20 µL	100 nM	Forward Primer: GAGGTACAAGAAGTATTTGC
• Assay 4 Forward (10 µM)	0.60 µL	300 nM	Reverse Primer: GCTTCCGGTGCATAAAG
• Assay 4 Reverse (10 µM)	0.60 µL	300 nM	Probe : [CAL Fluor Red 610]-TGCATATTATATCGAGCCACAGCATCG-[BHQ-2]
• Assay 4 Probe (10 µM)	0.20 µL	100 nM	
Total:	20.0 µL		<i>anthracis</i> -Specific – pXO2 – Biosynthesis Protein A
			Forward Primer: CTGGGTCATACTTAACGA
			Reverse Primer: TGCTACTCTTGGATTTACAG
			Probe : [Quasar 670]-CCTGGTTGTTCTTTCGTTGCAATAGC-[BHQ-2]

## Results:



All three categories of primer were used to amplify the same quadruplexed assay across the 1 in 4 dilution series of three different targets, while the fourth target was kept constant.

Viewing the resulting amplifications reveals subtle differences but no constant trends, particularly between the cartridge-purified and desalted varieties.

HPLC purification produces amplifications that reach a slightly higher fluorescence, but this is also subtle and doesn't justify the introduction of a more-stringently purified product.

## Conclusions:

The subtle differences between the primers are not statistically significant and are believed to perform equivalently in the context of this quadruplexed 5' nuclease assay. This particularly stringent assay requires an ensemble of eight different primers; problems with any one would be revealed upon one of the four channels. It is strongly believed that these results remain valid for other qPCR assays and applications.