

CASE STUDY: Use of Enzymatics' Superior Enzyme Quality and Analytics to Fix a Client's Reagent

Summary: A major producer of reagents for molecular biology applications was having problems with its Reagent Mix. The production of a key enzyme in the Reagent Mix was being outsourced to one of the world's most used enzyme Manufacturers. The problem the Client faced was that the Reagent Mix was intermittently failing in-house functional QC testing. The Client suspected that the Manufacturer's enzyme was the source of the inconsistent performance and engaged Enzymatics to employ its unique analytical tests to diagnose and solve the enzyme performance issue.

Performance Problem

In 2007 one of the world's leading providers of kits and reagents for molecular biology engaged Enzymatics regarding a problematic enzyme in a Reagent Mix which they market. The Client produces many of its own components and outsources others to outside companies. One of the major sources of the Client's revenue, a Reagent Mix involved in DNA amplification (RM-1), was intermittently failing internal functional testing. They narrowed the source of the problem to an enzyme that was being produced by one of the world's leading Manufacturers (Company X). Despite the fact that the enzyme continuously passed Company X's QC inspections, various lots were giving variable results when run in a RM-1 functional QC test. Neither Company X nor the Client understood the nature of the inconsistency. The Client needed to fix the problem to ensure it could keep up with customer demand for RM-1.

THE NEW STANDARD IN ENZYME PRODUCTION

Enzymatics' Superior Analytics

Enzymatics has developed superior approaches to analyzing enzyme quality that far surpass previous industry standards. The Client supplied Enzymatics with four lots of the enzyme from Company X. Enzymatics' scientists first ran an SDS PAGE gel stained with Silver Nitrate on the enzyme. This method is 50X more sensitive than Coomassie staining which is typically used by enzyme manufacturers as a purity test. The gel clearly showed (see Figure 3) both substantial and inconsistent levels of protein contamination across several of Company X's lots of the enzyme. This is notable but was not enough to explain the enzyme's inconsistent lot-to-lot performance in the RM-1 functional test.

Digging Deeper: Examination of Nuclease Contamination

One of Enzymatics' unique QC tests involves a proprietary test for nuclease contamination. This single-stranded nuclease assay monitors the degradation of DNA molecules and offers three orders of magnitude (1,000X) higher sensitivity than methods used by other enzyme providers for the same purpose. Enzymatics ran the test on these four lots and compared the results with three lots of its own enzyme. The results (shown in Figure 1) clearly demonstrate that Company X

has problems both with nuclease contamination and lot-to-lot consistency (higher bars indicate greater contamination). This was a clear indication that contamination in Company X's enzyme could be a contributing factor in the irregular functional outcome of RM-1 tests. None of these lots of the enzyme would have met Enzymatics' release criteria for nuclease contamination while all three lots of Enzymatics' enzyme showed negligible to no contamination.

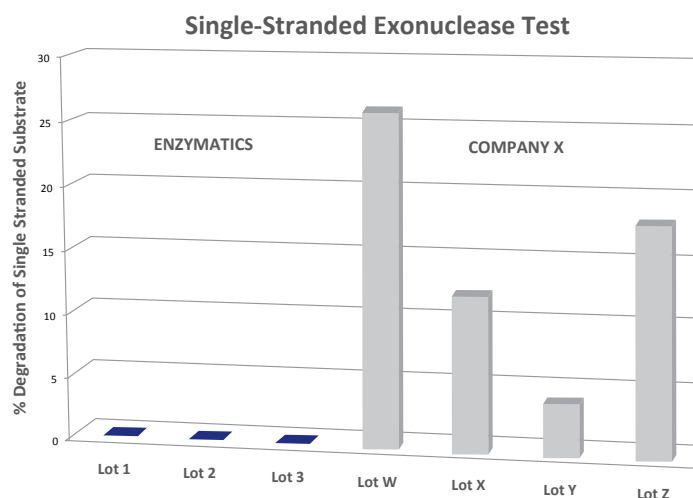


Figure 1: Results of nuclease contamination test using a proprietary single-stranded exonuclease test.

The Final Piece of the Puzzle: DNA Contamination

A final analysis was performed to confirm that Company X's enzyme was behind RM-1's functional performance problem. Enzymatics analyzed the DNA contamination level of several lots of the enzyme from both companies. The test measures the relative level of nucleic acid contamination using qPCR against the E.coli 16S rRNA subunit, the most common DNA sequence in the host organism, to determine the level of residual DNA present. The results, as illustrated in Figure 2 on the next page, further confirmed the lot-to-lot variability of Company X's enzyme and pointed to substantial levels of DNA contamination in the various lots. By contrast, Enzymatics' three lots of the enzyme show both consistent and negligible levels of DNA contamination.

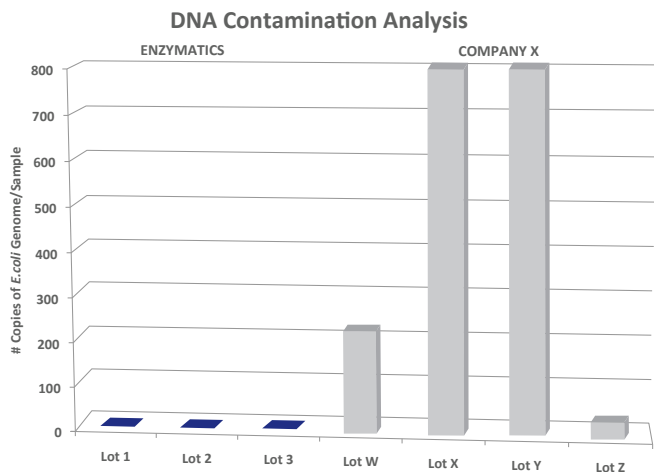
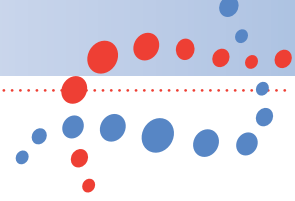


Figure 2: DNA contamination measured in 3 lots of the Enzymatics enzyme and 4 lots of the enzyme from Company X.

Putting it all together: Confirming the Culprit

The compilation of all three analyses is depicted in Figure 3 in which the inconsistency and impurity levels of Company X's enzyme are clearly visible. This lends credence to the Client's conclusion that the enzyme was the cause of the erratic performance seen in their functional test of RM-1. The lot-to-lot uniformity and purity of Enzymatics' enzyme is clearly seen in Figure 3.

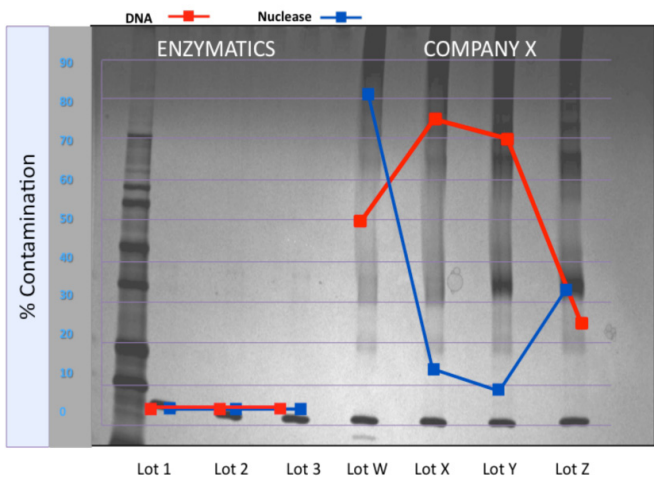


Figure 3: Comparison of Enzymatics' enzyme with that of Company X for purity, DNA contamination, and nuclease contamination.

Solving the Problem and Lowering the Cost

When the enzyme produced by Enzymatics was incorporated into RM-1, the functional test performance and lot-to-lot consistency soared due to its superior quality. Of substantial financial benefit to the Client is that the Enzymatics enzyme is priced at 80% below that of Company X's enzyme. Enzymatics' intellectual property, advanced technology platform and unique business model enables the company to produce ISO 13485:2003 compliant enzymes at a fraction of the cost of other companies' research grade materials.

Figure 4 shows a cost per unit comparison of six of the more commonly used enzymes across the five largest vendors of enzymes in the world as compared to Enzymatics. The list prices for Enzymatics' enzymes are up to 90% lower than the list prices of the five most commonly used vendors.

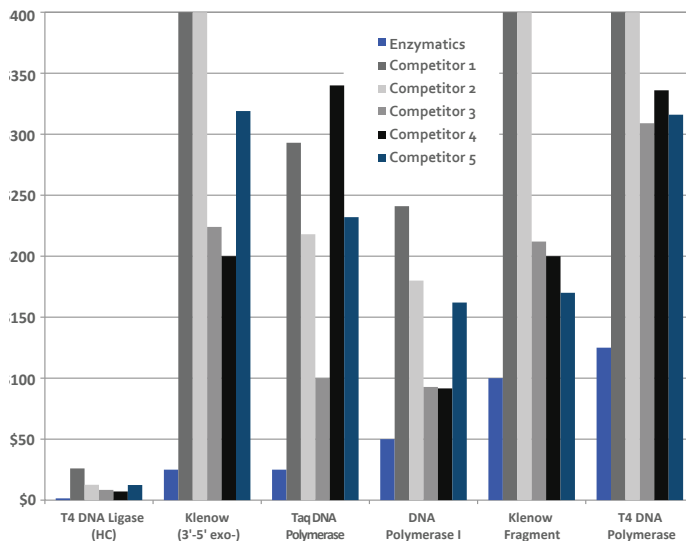


Figure 4: Price comparison of Enzymatics' enzymes to market leaders. All prices are list price per unit enzyme.

Conclusion

Enzymatics' unique combination of high quality enzymes, price and service made it the clear cut choice for this client, as well as a global array of commercial genetic analysis companies. In addition to marketing dozens of Ultra Pure Enzymes, Enzymatics builds long-term contract research, manufacturing, and supply-chain management partnerships with organizations engaged in commercial molecular biology applications.

FOR MORE INFORMATION

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