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# ProteinSEQ™ Protein Quantitation System

Bringing the Power of qPCR to Protein Quantitation

The world leader in serving science

# Rapid molecular methods for pharmaceutical manufacturing

## Product Safety

Microbial Identification and Detection



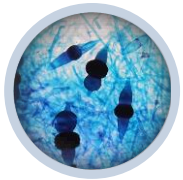
*DNA Sequencing*

## Product Quality

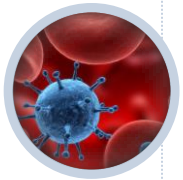
Impurity Analysis

*Real-Time PCR*

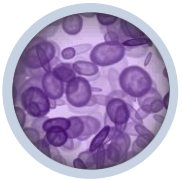
Bacterial ID



Fungal ID



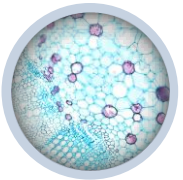
Virus Detection



Mycoplasma

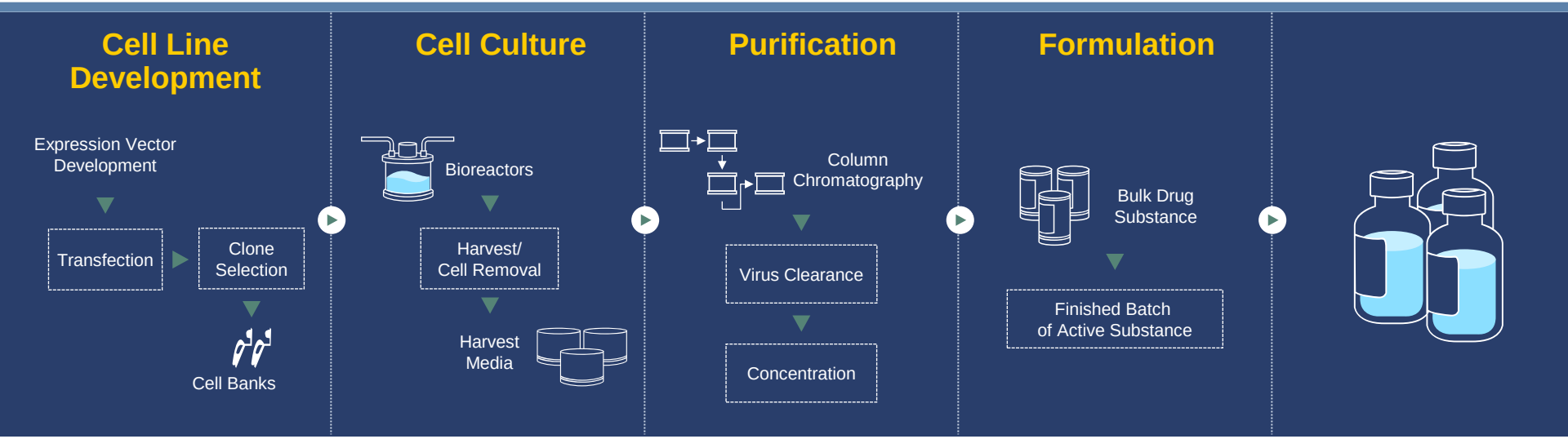
Residual DNA

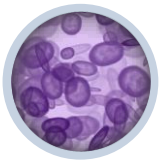
Protein A



Custom HCP

# Analytical Solutions for BioProduction



MycoSEQ™ Mycoplasma Detection System

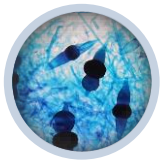
ViralSEQ™ Virus Detection System

resDNASEQ™ Residual DNA Quantitation System

NEW

ProteinSEQ™ HCP Quantitation System

ProteinSEQ™ Protein A Quantitation System (Arrives in 2015)



Bacterial & Fungal Identification



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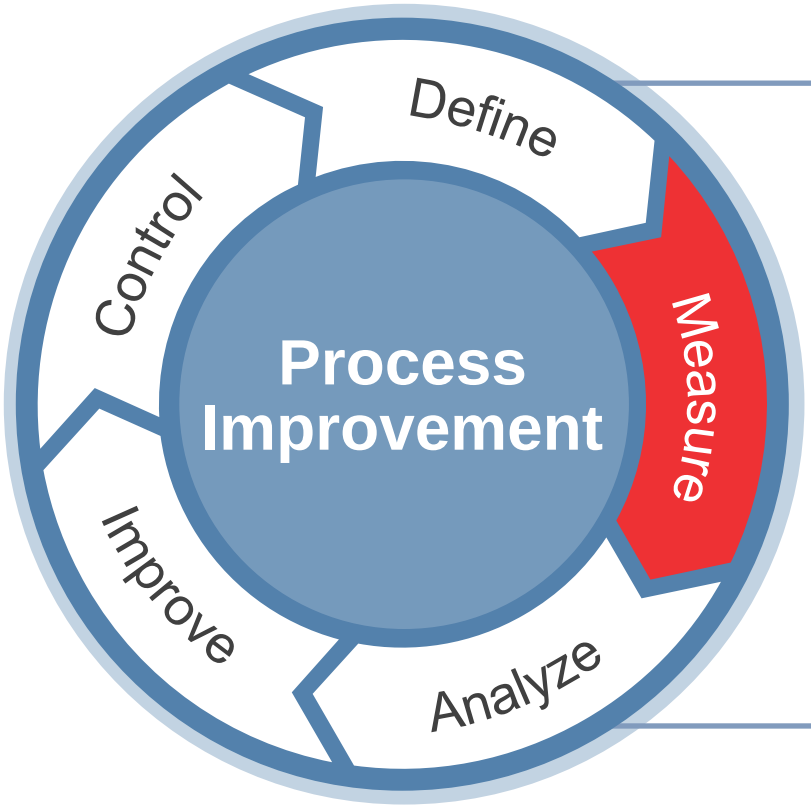
# Process Improvement Requires High Performance Analytics




*Measurement is the*

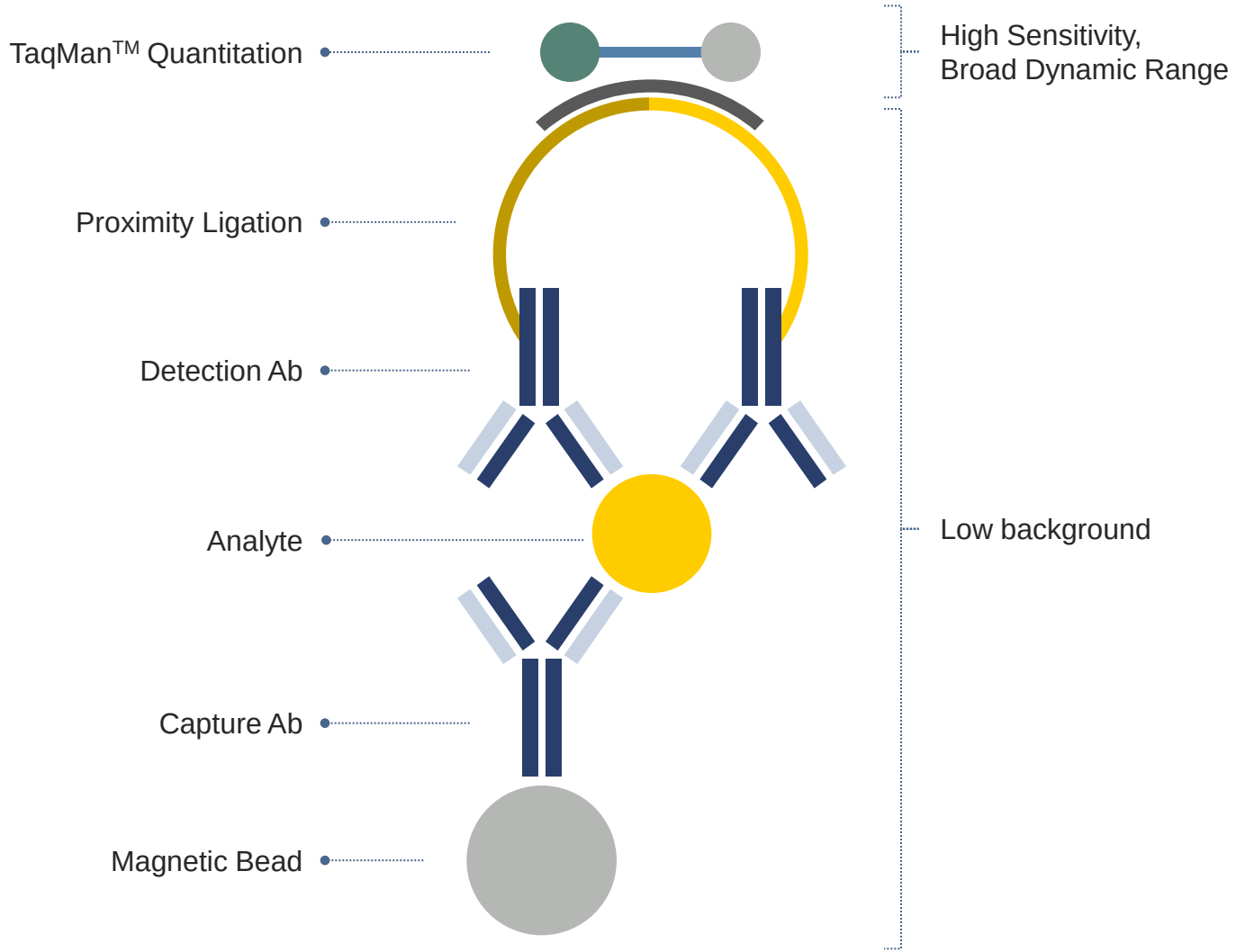
***first step***

*that leads to control and eventually to improvement.*

*If you can't measure something, you can't understand it.*
*If you can't understand it, you can't control it.*
*If you can't control it, you can't improve it.*

—H. James Harrington

# ProteinSEQ™ System Core Technology



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# Key Features for ProteinSEQ™ Quantitation System

4 Log
Dynamic Range

High
Sensitivity

Robust Dilution
Linearity

Robust
Efficiency

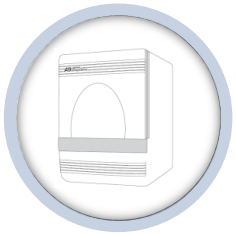
Semi-
Automation

Support
Network

# ProteinSEQ™ Protein Quantitation System

## ProteinSEQ™

Host Cell Protein Quantitation Kit



## Applied Biosystems™ 7500 FAST

Real-Time PCR Magnetic Particle Processor

## MagMAX™

Express-96 Instrument


## AccuSEQ™

Software



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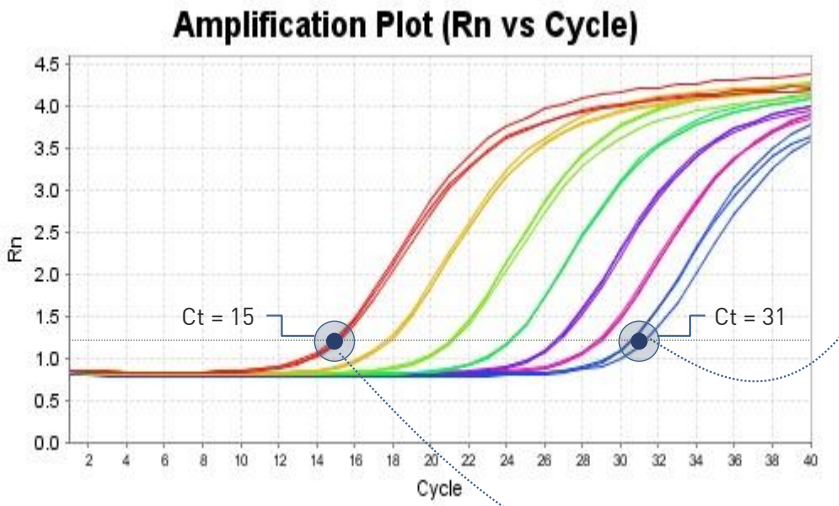
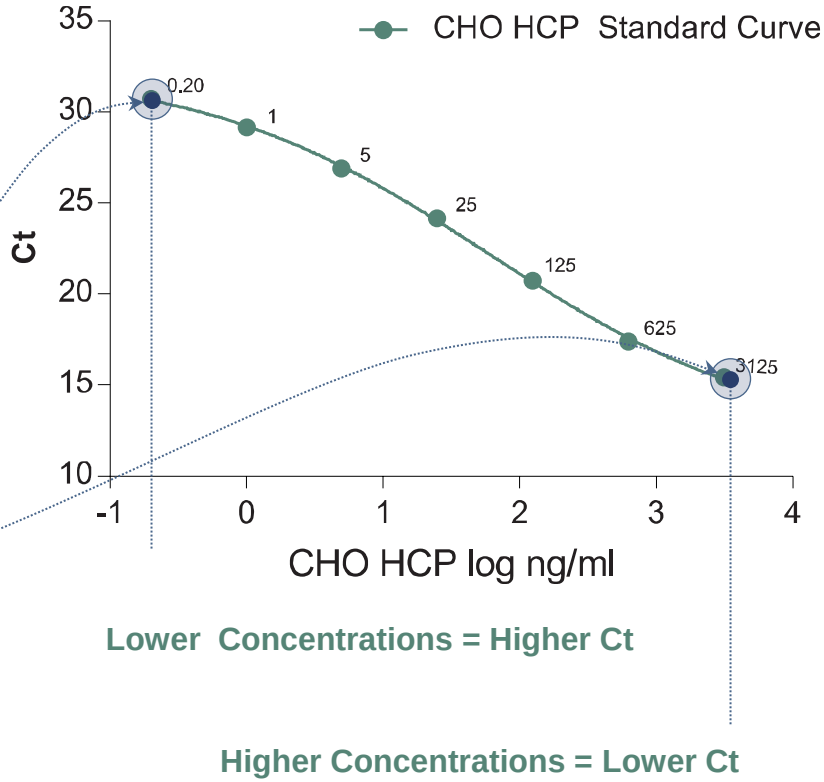
# Quantitation Based on a qPCR Amplification Threshold




**Lower Concentrations = Higher Ct**

**Higher Concentrations = Lower Ct**



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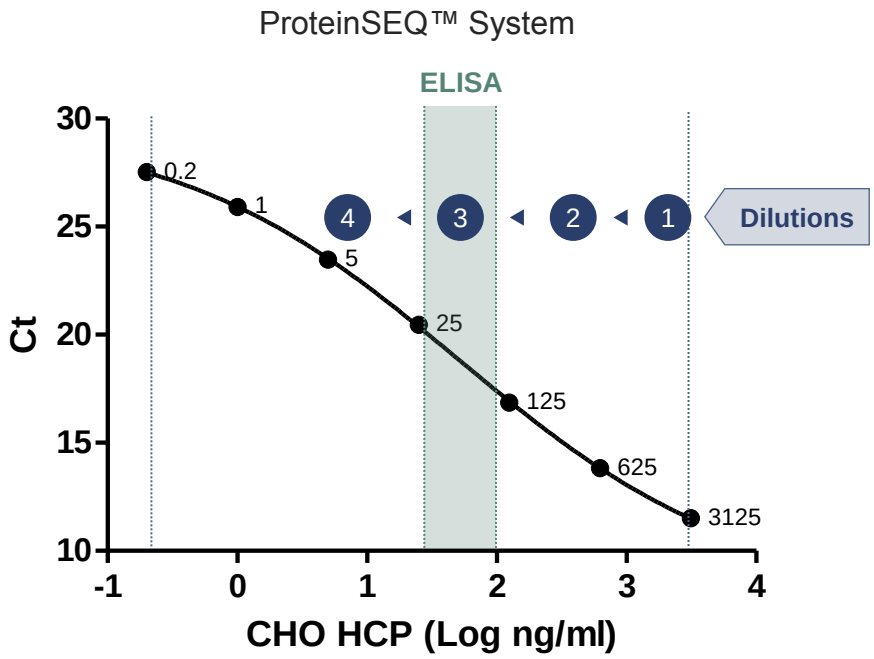
# Broad Dynamic Range: High Quality Data




- Helps reduce dilutions
- Helps reduce cost per data point
- Helps reduce hands-on time
- Enables faster time-to-result

- **Higher quality data than ELISA with the same antibody pool**
- **The platform, not just the antibody, can limit assay performance**



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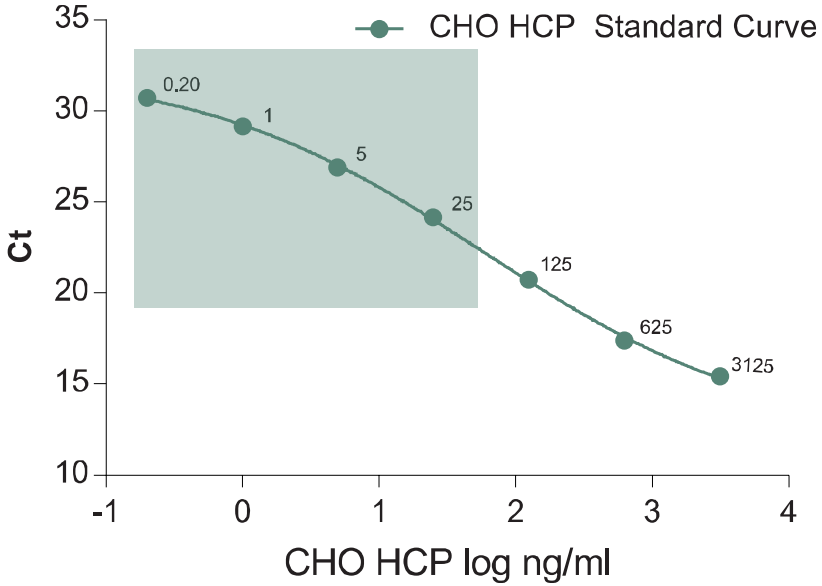
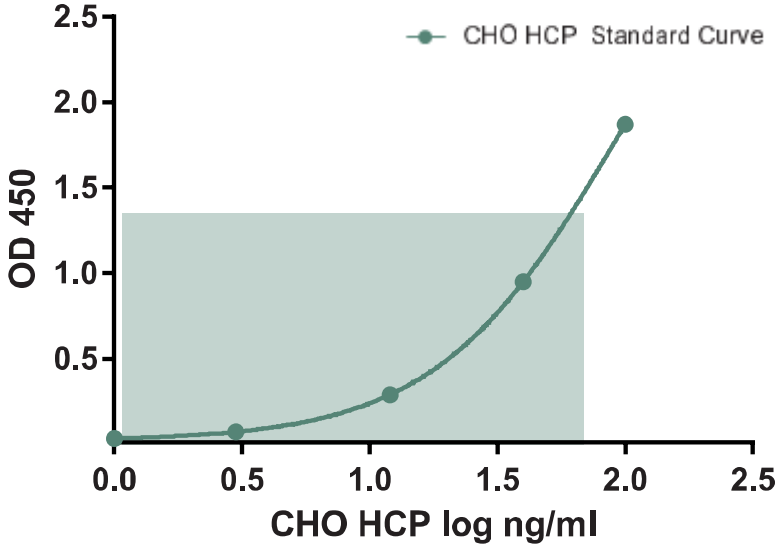
# Higher Sensitivity: Improves Process Characterization

## ProteinSEQ™ System




## Commercial ELISA




**ProteinSEQ™ System retains excellent curve shape to 0.2 ng/mL**



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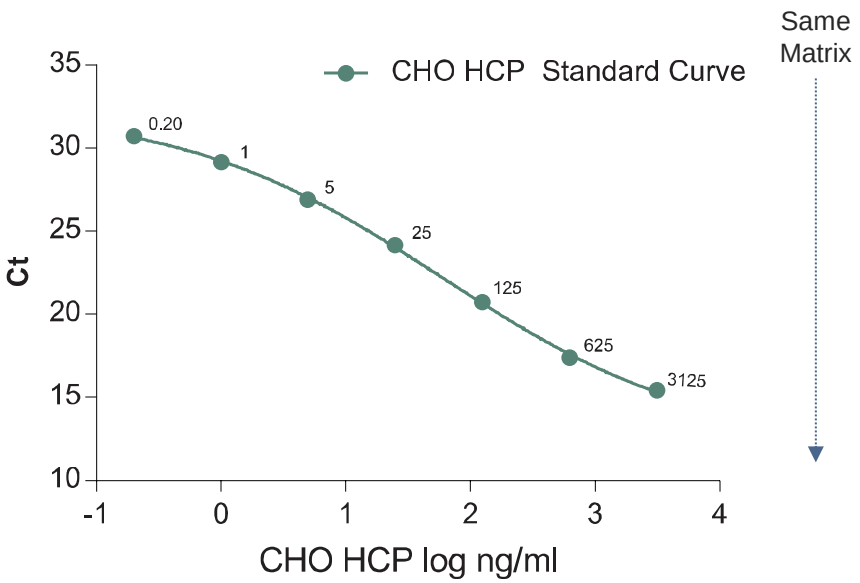
# Superior Dilution Linearity and Efficiency

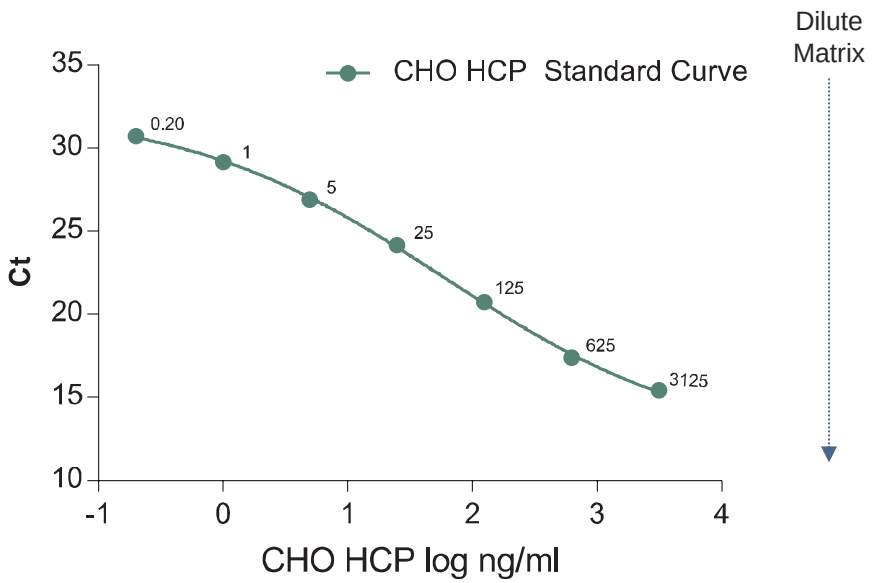
## Performance across **constant matrix**




*Answers the question:*

How will the assay perform when an analyte is measured in a specific matrix at a specific concentration?

## Performance across **changing matrix**

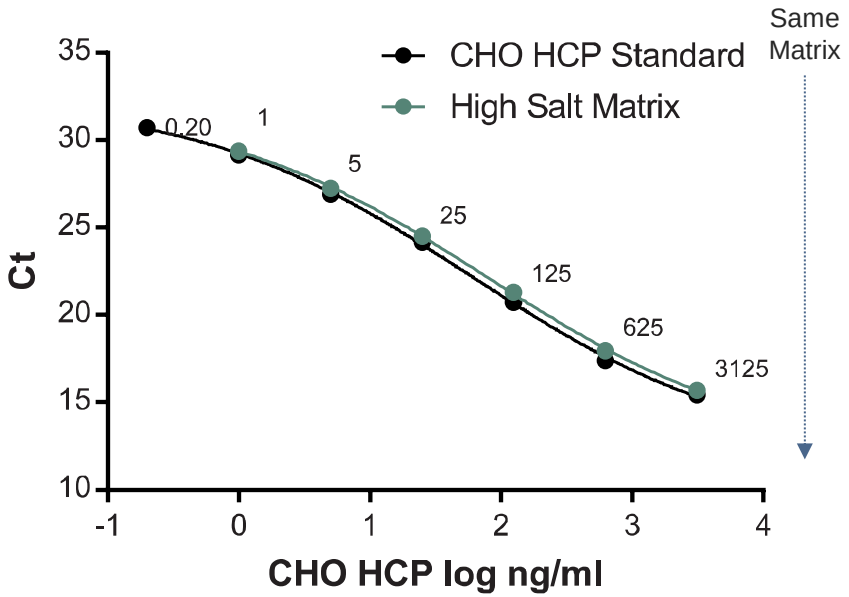



*Answers the question:*

How will the assay perform when the matrix changes over a series of dilutions?

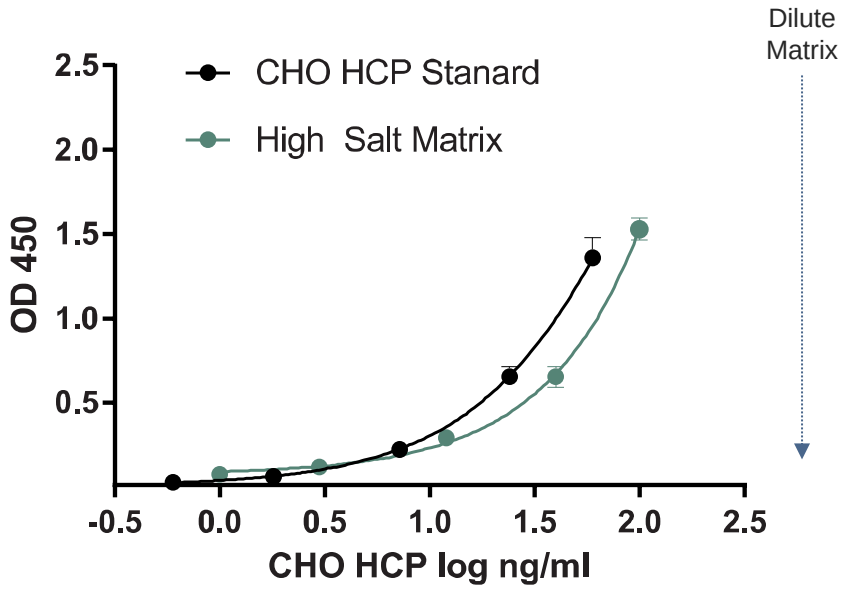
# Evaluation of Efficiency and Dilution Linearity

Measure performance across dynamic range with **constant matrix**




## Efficiency

Measure performance across dynamic range with **changing matrix**




## Dilution Linearity



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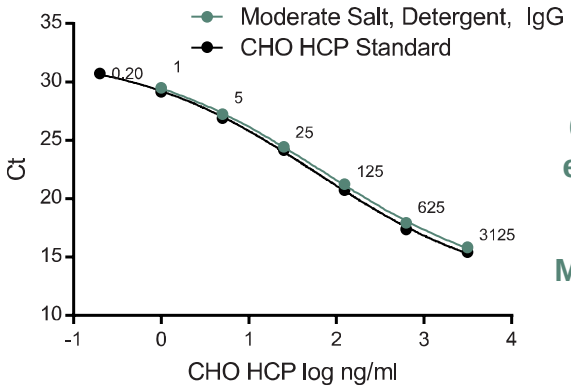
# Highly Efficient Quantitation with High Salt Matrices

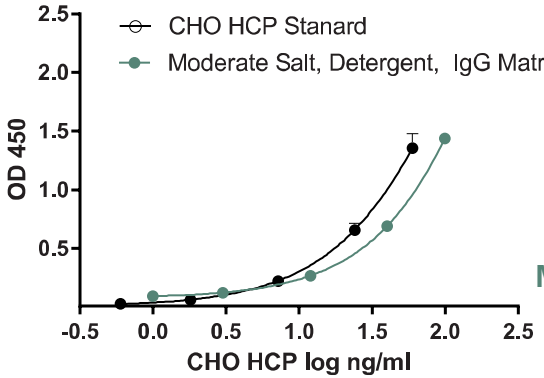
## 800 mM NaCl, 20 mM NaPO4, pH 7.5,10 mg/mL human IgG / 4 X Dilution




**6 of 6 results with efficiency 70-130% and < 20%CV**

**Matrix specific LOQ = 4*1= 4 ng/mL**

| Input | Result | Efficiency | CV% |
|---|---|---|---|
| 3125 | 2266.13 | 71.74 | 1.29 |
| 3125 | 2209.89 | | |
| 3125 | 2249.61 | | |
| 625 | 505.57 | 80.65 | 1.12 |
| 625 | 508.79 | | |
| 625 | 497.77 | | |
| 125 | 89.12 | 74.15 | 5.38 |
| 125 | 90.55 | | |
| 125 | 98.38 | | |
| 25 | 18.91 | 78.63 | 3.98 |
| 25 | 19.59 | | |
| 25 | 20.47 | | |
| 5 | 4.23 | 86.07 | 5.54 |
| 5 | 4.11 | | |
| 5 | 4.57 | | |
| 1 | 0.8575 | 87.53 | 16.75 |
| 1 | 0.7384 | | |
| 1 | 1.03 | | |




**2 of 5 results with efficiency 70-130% and < 20%CV**

**Matrix specific LOQ = 4*12= 48 ng/mL**

| Input | Result | Efficiency | CV% |
|---|---|---|---|
| 100 | 74.21609 | 73.02 | 6.12 |
| 100 | 76.76676 | | |
| 100 | 68.07426 | | |
| 40 | 25.31818 | 66.76 | 9.53 |
| 40 | 29.64259 | | |
| 40 | 25.15164 | | |
| 12 | 12.69266 | 100.49 | 6.71 |
| 12 | 12.33667 | | |
| 12 | 11.14765 | | |
| 3 | 5.22759 | 170.88 | 5.73 |
| 3 | 4.795592 | | |
| 3 | 5.356152 | | |
| 1 | 3.227993 | 322.55 | 11.52 |
| 1 | 2.852625 | | |
| 1 | 3.595807 | | |

**ProteinSEQ™ kits demonstrate accurate quantitation in high salt / Matrix specific LOQ 12-100X lower than ELISA**



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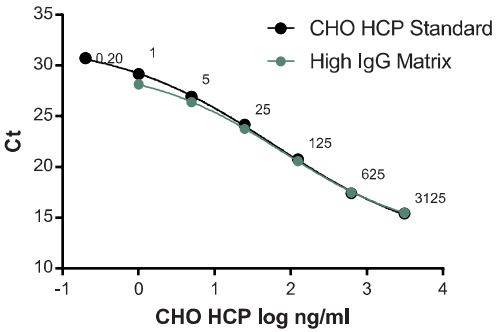
# Highly Efficient Quantitation with Complex Samples

100 mM NaPO4, pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.25% Tween-20, 15 mg/mL human IgG

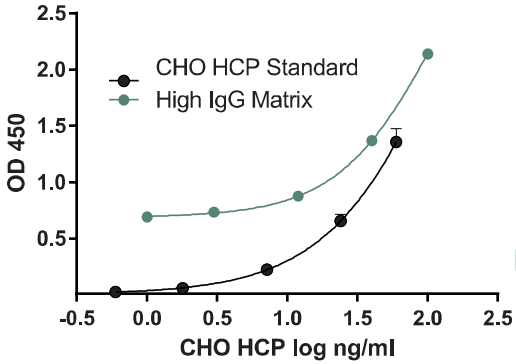
4X Dilution




**5 of 6 results with efficiency 70-130% and < 20%CV**

**LOQ = 1*4= 4 ng/mL**

| Input | Result | Efficiency | CV% |
|---|---|---|---|
| 3125 | 1964.12 | 65.34 | 6.83 |
| 3125 | 2202.93 | | |
| 3125 | 1958.96 | | |
| 625 | 500.60 | 81.39 | 7.55 |
| 625 | 550.52 | | |
| 625 | 474.99 | | |
| 125 | 96.28 | 74.93 | 8.13 |
| 125 | 99.62 | | |
| 125 | 85.08 | | |
| 25 | 19.64 | 81.40 | 5.34 |
| 25 | 21.60 | | |
| 25 | 19.81 | | |
| 5 | 4.42 | 87.47 | 4.44 |
| 5 | 4.54 | | |
| 5 | 4.16 | | |
| 1 | 0.7979 | 78.75 | 8.29 |
| 1 | 0.8469 | | |
| 1 | 0.7176 | | |




**2 of 5 results with efficiency 70-130% and < 20%CV**

**LOQ =4*12=48 ng/mL**

| Input | Result | Efficiency | CV% |
|---|---|---|---|
| 100 | 69.12405 | 66.89 | 7.47 |
| 100 | 70.3869 | | |
| 100 | 61.17376 | | |
| 40 | 28.0132 | 70.47 | 4.27 |
| 40 | 29.46998 | | |
| 40 | 27.08011 | | |
| 12 | 11.10794 | 93.23 | 1.23 |
| 12 | 11.34615 | | |
| 12 | 11.10794 | | |
| 3 | 5.611974 | 175.65 | 5.86 |
| 3 | 5.012284 | | |
| 3 | 5.184636 | | |
| 1 | 3.867462 | 392.60 | 8.10 |
| 1 | 4.269155 | | |
| 1 | 3.641318 | | |

**ProteinSEQ™ kits demonstrate accuracy in complex matrices whereas ELISA doesnot**

**Matrix specific LOQ 12-100X lower than ELISA**



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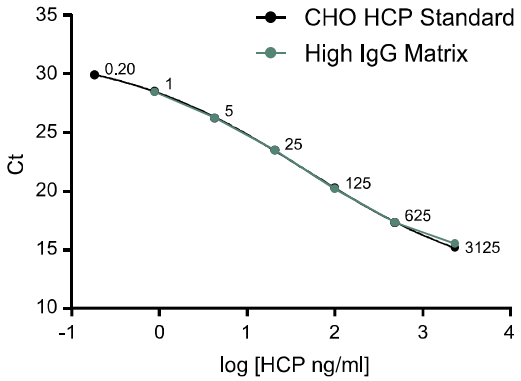
# Highly efficient with High IgG Matrix

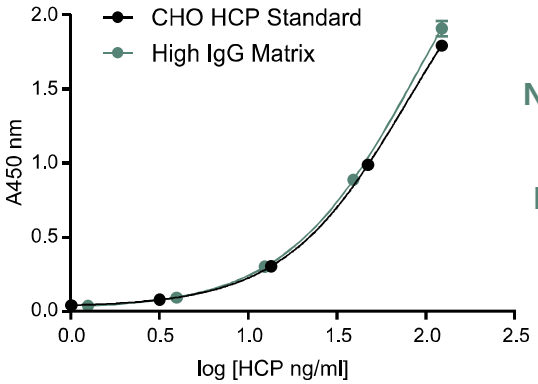
## 100 mM NaPO4, 100 mg/mL human IgG




**4 of 6 results with efficiency 70-130% and < 20%CV**

**Matrix specific LOQ = 25*8= 200 ng/mL**

| Input | Result | Efficiency% | CV% |
|---|---|---|---|
| 3125 | 2655.25 | 85.75 | 1.28 |
| 3125 | 2718.96 | | |
| 3125 | 2664.66 | | |
| 625 | 626.42 | 105.44 | 6.52 |
| 625 | 642.84 | | |
| 625 | 707.72 | | |
| 125 | 125.82 | 103.03 | 3.96 |
| 125 | 125.86 | | |
| 125 | 134.67 | | |
| 25 | 27.83 | 113.40 | 1.64 |
| 25 | 28.49 | | |
| 25 | 28.73 | | |
| 5 | 7.14 | 143.40 | 2.05 |
| 5 | 7.04 | | |
| 5 | 7.33 | | |
| 1 | 1.85 | 240.00 | 20.32 |
| 1 | 2.57 | | |
| 1 | 2.78 | | |




**No valid results with 70-130% efficiency**

**Matrix specific LOQ = > 800 ng/mL**

| Input | Result | Efficiency% | CV% |
|---|---|---|---|
| 100 | 137.8936 | 129.14 | 7.74 |
| 100 | 131.2655 | | |
| 100 | 118.2532 | | |
| 40 | 62.45045 | 156.45 | 2.16 |
| 40 | 61.29456 | | |
| 40 | 63.99461 | | |
| 12 | 39.98194 | 304.14 | 8.69 |
| 12 | 33.77962 | | |
| 12 | 35.72802 | | |
| 3 | 29.68579 | 1005.76 | 6.28 |
| 3 | 32.26471 | | |
| 3 | 28.56804 | | |
| 1 | 29.64259 | 2843.73 | 13.30 |
| 1 | 31.47141 | | |
| 1 | 24.19804 | | |

**ProteinSEQ™ kits demonstrated accuracy with high IgG whereas ELISA did not** / **Matrix specific LOQ > 4X lower than ELSIA**



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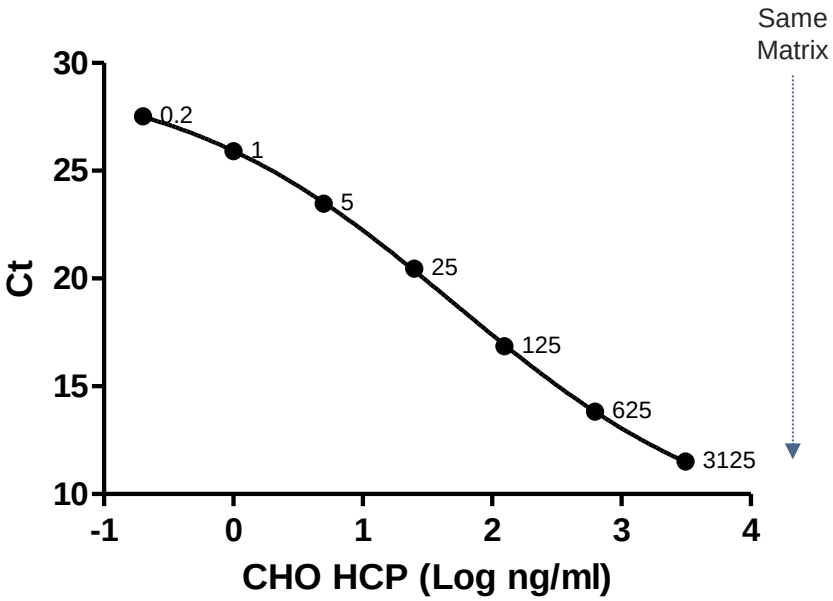
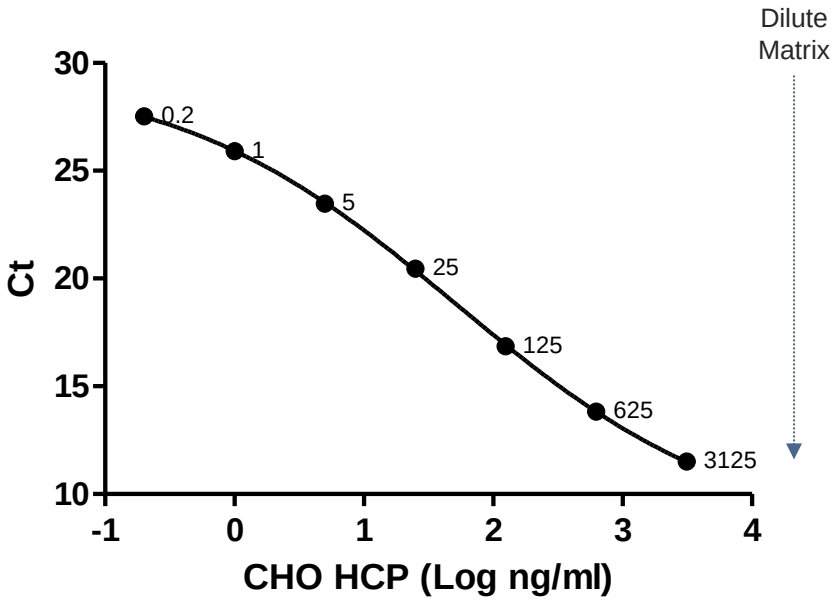
# Evaluation of Efficiency and Dilution Linearity

Measure performance across dynamic range with **constant matrix**




**Efficiency**

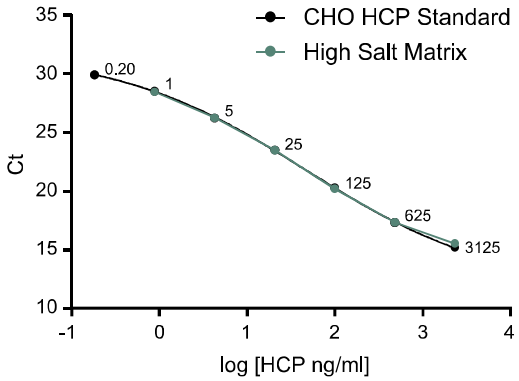
Measure performance across dynamic range with **changing matrix**




**Dilution Linearity**

# Consistent in Dilution Linearity with High Salt Matrix

## 800 mM NaCl, 20 mM NaPO4, pH 7.5,10 mg/mL human IgG

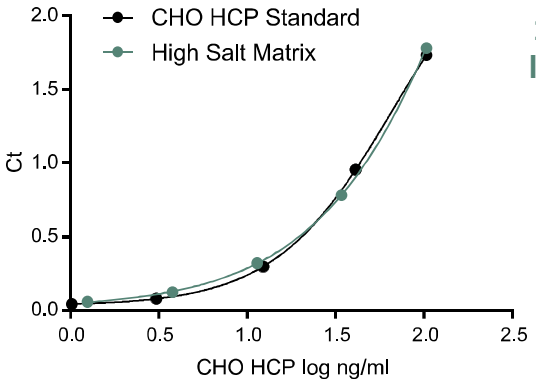



**13.1 %CV over 3.5 logs dynamic range**

**6 of 6 results with efficiency 70-130% and < 20%CV**

**Matrix specific dynamic range = 3.49 logs**

| Input | Dilution | Result | Corrected Result | Avg. | Linearity |
|---|---|---|---|---|---|
| 3125 | 4 | 2304 | 9216 | 73.04 | 13.1% over 3.5 logs |
| 3125 | | 2260 | 9040 | | |
| 3125 | | 2283 | 9132 | | |
| 625 | 20 | 596 | 11920 | 102.68 | |
| 625 | | 671 | 13420 | | |
| 625 | | 658 | 13160 | | |
| 125 | 100 | 131 | 13100 | 103.30 | |
| 125 | | 130 | 13000 | | |
| 125 | | 126 | 12600 | | |
| 25 | 500 | 22.3 | 11150 | 101.60 | |
| 25 | | 26.0 | 13000 | | |
| 25 | | 25.2 | 12600 | | |
| 5 | 2500 | 4.78 | 11950 | 102.55 | |
| 5 | | 5.18 | 12950 | | |
| 5 | | 5.52 | 13800 | | |
| 1 | 12500 | 1.07 | 13375 | 96.67 | |
| 1 | | 1.13 | 14125 | | |
| 1 | | 0.91 | 11375 | | |




**16.25 %CV over 1.4 logs dynamic range**

**4 of 5 results with efficiency 70-130% and < 20%CV**

**Matrix specific dynamic range = .95 logs**

| Input | Dilution | Result | Corrected Result | Avg. | Linearity |
|---|---|---|---|---|---|
| 100 | 4 | 112.60 | 450.4 | 104.14 | 16% over 1.4 logs |
| 100 | | 98.25 | 393 | | |
| 100 | | 101.59 | 406.36 | | |
| 33.33 | 12 | 32.91 | 394.92 | 92.61 | |
| 33.33 | | 29.76 | 357.12 | | |
| 33.33 | | 29.93 | 359.16 | | |
| 11.11 | 36 | 12.25 | 441 | 109.22 | |
| 11.11 | | 12.87 | 463.32 | | |
| 11.11 | | 11.28 | 406.08 | | |
| 3.7 | 108 | 5.57 | 601.56 | 112.57 | |
| 3.7 | | 3.65 | 394.2 | | |
| 3.7 | | 3.27 | 353.16 | | |
| 1.23 | | | 0 | 134.62 | |
| 1.23 | 324 | 1.66 | 537.84 | | |
| 1.23 | | | 0 | | |

**ProteinSEQ™ kits are more linear over a 2X greater dynamic range in high salt**



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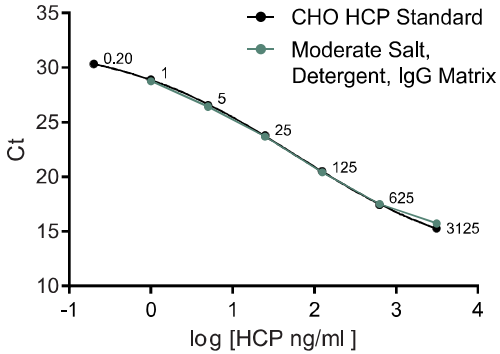
# Consistent in Dilution Linearity with Complex Samples

## 100 mM NaPO4, pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.25% Tween-20, 15 mg/mL human IgG

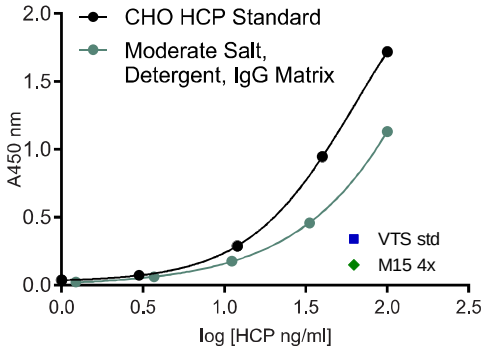



**%CV = 18% over 3.4 logs dynamic range**

**5 of 6 results with 70-130% efficiency**

**Matrix specific dynamic range = 3.49 logs**

| Input | Result | Avg. | CV% |
|---|---|---|---|
| 3125 | 2347 | 67.24 | 11.14 |
| 3125 | 2075 | | |
| 3125 | 1882 | | |
| 625 | 629 | 101.06 | 8.97 |
| 625 | 690 | | |
| 625 | 577 | | |
| 125 | 136 | 103.90 | 8.01 |
| 125 | 136 | | |
| 125 | 118 | | |
| 25 | 25.18 | 109.43 | 10.24 |
| 25 | 27.35 | | |
| 25 | 24.27 | | |
| 5 | 6.04 | 111.11 | 10.67 |
| 5 | 6.28 | | |
| 5 | 4.90 | | |
| 1 | 0.98 | 108.35 | 9.79 |
| 1 | 1.17 | | |
| 1 | 1.18 | | |
| **Average** | | | **18.0** |



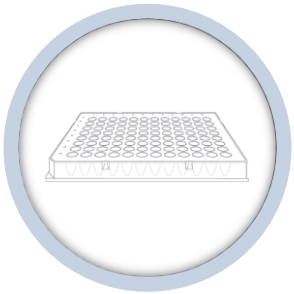

**No valid results with 70-130% efficiency**

**Matrix specific dynamic range = NA**

| Input | Result | Avg. | CV% |
|---|---|---|---|
| 100 | 47.88 | 49.05 | 6.71 |
| 100 | 52.77 | | |
| 100 | 46.51 | | |
| 33.33 | 17.83 | 53.43 | 2.64 |
| 33.33 | 18.26 | | |
| 33.33 | 17.32 | | |
| 11.11 | 6.42 | 60.36 | 4.32 |
| 11.11 | 7.00 | | |
| 11.11 | 6.71 | | |
| 3.7 | 1.17 | 33.38 | 5.08 |
| 3.7 | 1.30 | | |
| 3.7 | 1.24 | | |
| 1.23 | | 0.00 | N/A |
| 1.23 | | | |
| 1.23 | | | |
| **Average** | | | **21.49** |

**ProteinSEQ™ kits demonstrate accurate quantitation across serial dilutions in a complex matrix whereas ELISA does not**

# Semi-Automated ProteinSEQ™ System Workflow

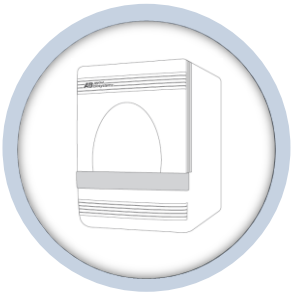


**Sample Prep**

.5-1 hour

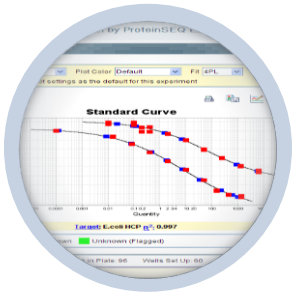
**Automated Processing**

2 hours walk-away



**qPCR**

1 hour




**Data Analysis**

15-50 min

Capture ▸ Wash ▸ Wash ▸ Probes ▸ Wash ▸ Wash



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# Summary of ProteinSEQ™ System for HCP

## Wide Dynamic Range

4 Log dynamic range reduces dilutions and hands on time

## High Sensitivity

Lower LOQ provides more data for process improvement

## Robust Linearity

Reduces dilutions and simplifies data analysis

## Robust Efficiency

Accurate quantitation in complex matrices

## Semi-Automation

Walk-away processing of 96-well plates

## Support Network

Worldwide technical and validation support and custom capabilities

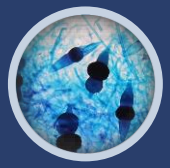
MicroSEQ™ System

AutoMate *Express*™ Instrument

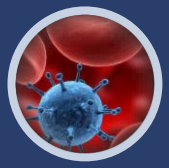
MagMAX™ Express-96

7500 Fast Real-Time PCR System

Bacterial ID



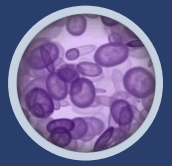
Fungal ID



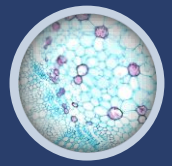
Virus Detection

Protein A

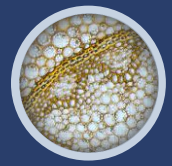
Residual DNA



Mycoplasma



HCP



BioBurden

## A Real-Time qPCR platform to detect a broad spectrum of common contaminants with optional automation

# Legal Disclaimer

**For Research Use Only. Not for use in diagnostic procedures.**

# *Appendix*

# ProteinSEQ™ System for HCP Requires 3 Epitopes per Antigen

## The Goal

To quantitate HCPs with the highest sensitivity and reproducibility

- ProteinSEQ™ kits are more sensitive and delivers reproducible values at lower concentrations than ELISA
- Higher quality data for downstream steps enables improved process monitoring and development

# HCPs can Have a Variable Number of Epitopes

HCPs can vary in their immunogenicity during preparation of the anti-HCP polyclonal antibody. Regardless of size, some HCPs will reactive strongly with the host animal and others will not.


## Crude HCP Sample

Contains hundreds to thousands of proteins with-all with different immunogenicity towards the host animal. Two are shown below

## Polyclonal Antibody

Diverse antibody species

## Antigen Recognition

Diverse ratio of HCP to antibody binding. The opaque HCP has two epitopes while the blue HCP has 8.

# Variable Epitopes on HCP Quantitation

All Ab based HCP assays actually count epitopes not mass. For this reason, the reported metric of “ng/mL” is not an accurate description of the actual measurement.

**Example:** If an HCP is very immunogenic it will generate many antibody species during preparation of the polyclonal antibody reagent in goat/sheep/rabbit/chicken. Removal of a small amount of this protein will lead to a large change in signal. The assay reports a large change in “mass” even though this was not the case.

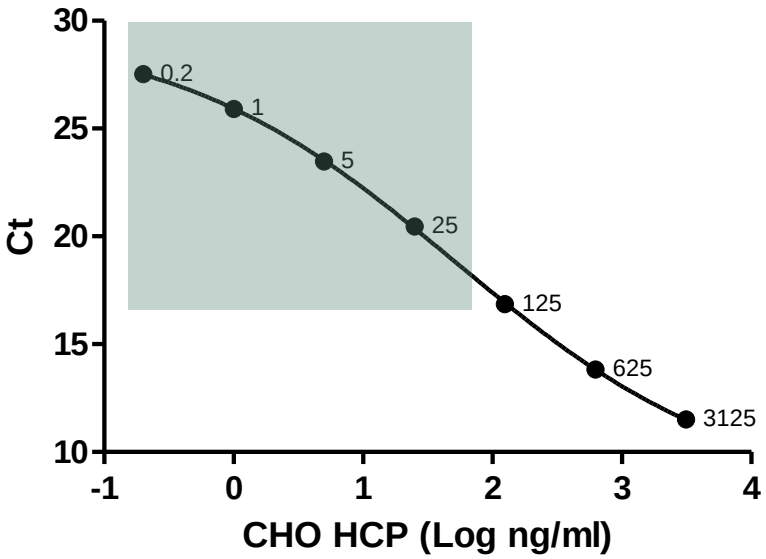

- Contains blue HCP with 8 epitopes and a opaque HCP with few 2 epitopes
- Proteins are present at equal concentration of 500 ng/mL
- HCP assay reports 1000 ng/mL

- Blue HCP is removed during purification
- Opaque HCP remains in product
- HCP assay reports 125 ng/mL remains in products
- Result would indicate 8Xdecrease in HCP (1000/125) mass even though it was really only 2X (1000/500)
- HCP assay OVERESTIMATES amount of HCP removed due to epitope variation by 4X (500 ng/mL/125 ng/mL)

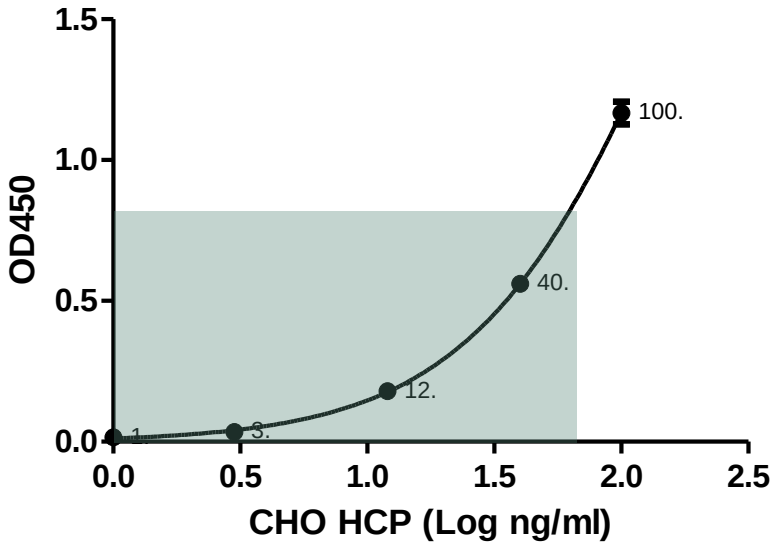
# Reduced Coverage Would Reduce Sensitivity

All ProteinSEQ™ System assays (to date) compared side-by-side with ELISA are MORE sensitive than ELISA indicating no significant proteins are missed

ProteinSEQ™ System




Commercial ELISA



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