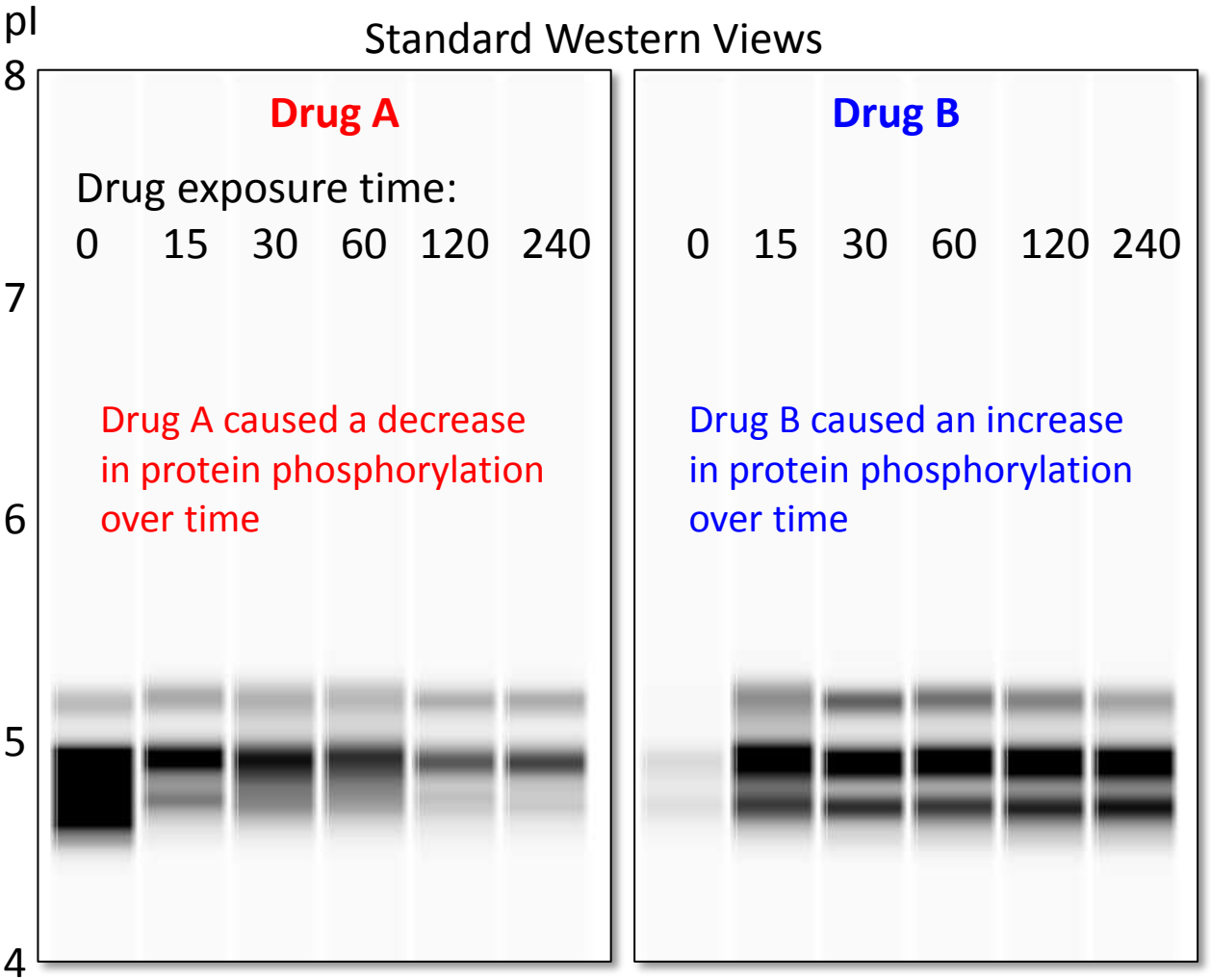
Simple Western™ Case Studies

- Drug Effect On Protein Phosphorylation
- Characterization Of Biotherapeutics
- Drug Response Measurement
- Identification Of Clinical Trial Patient Response
- Monoclonal Antibody Analysis
- Drug Response Time Course
- Calculating Concentration of Unknown Biomarker
- Optimization of Biomarker Recovery
- Knockdown Experiment Using siRNA

Drug Effect on Protein Phosphorylation

by Charge (IEF) Analysis

Analysis of two chemotherapeutics drugs (**A** & **B**) at a single concentration on cell culture cells over a time course treatment, 0-240 minutes, found dramatic changes in protein phosphorylation.

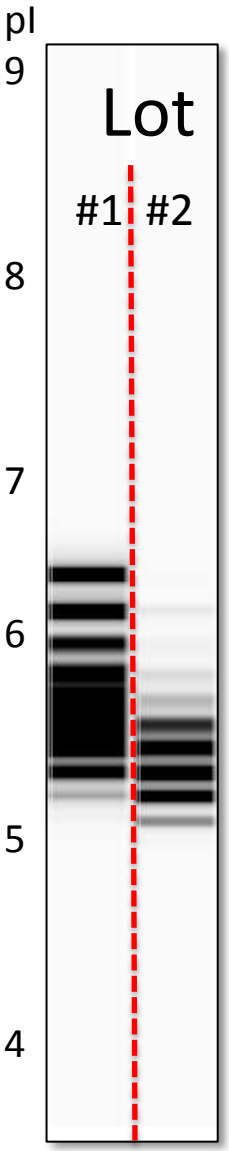


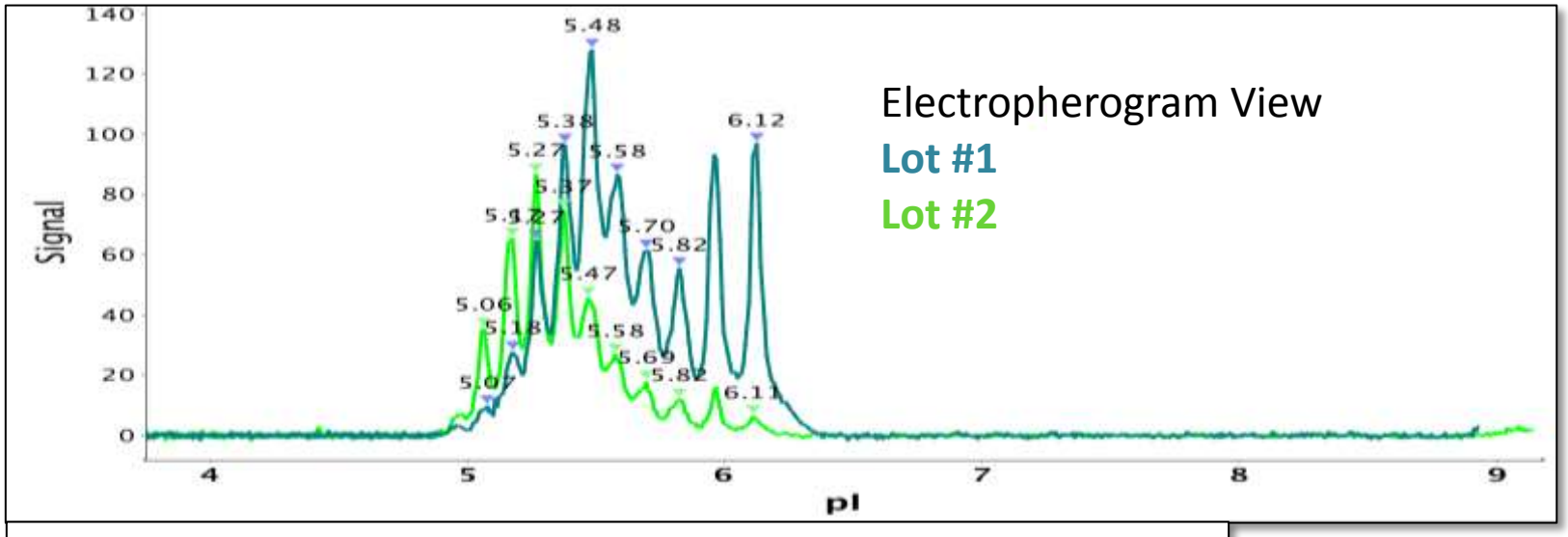
SBH DIAGNOSTICS™

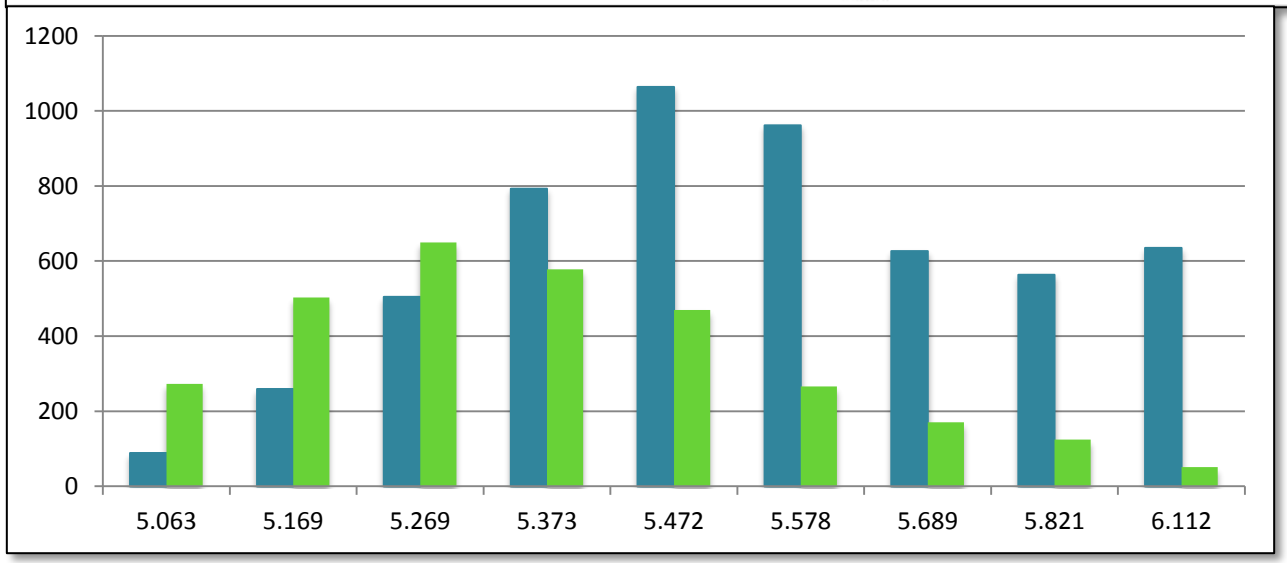
Characterization of Biotherapeutic production

Identification of changes in Post Translational Modifications of proteins by Charge (IEF) Simple Western

Analysis of two production lots





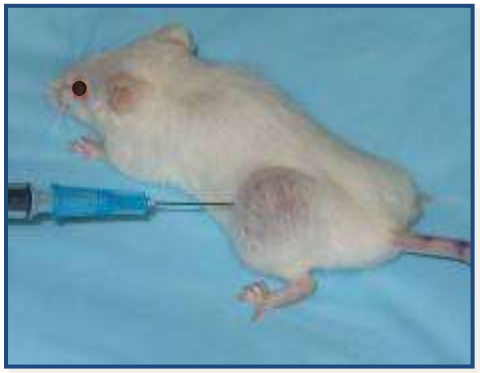


Quantitative measurement of individual peaks

SBH DIAGNOSTICS™

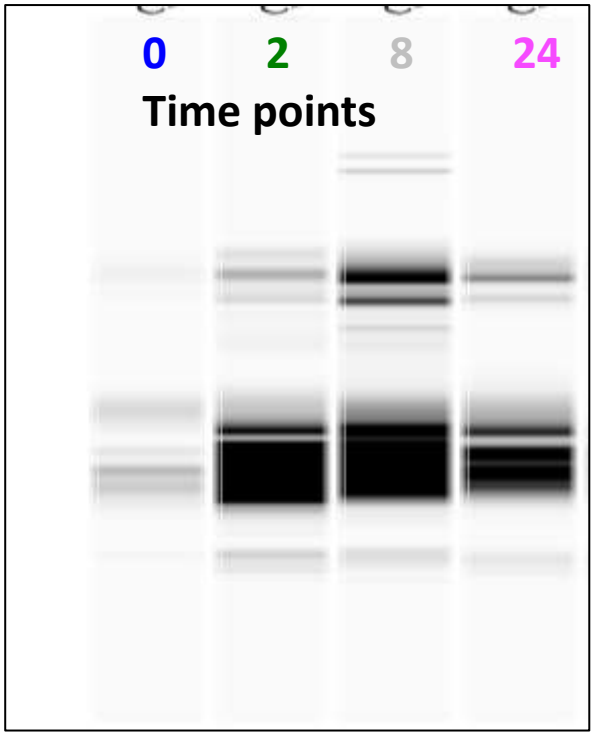
Drug Response Measurement

using Fine Needle Aspirates of animal tumors

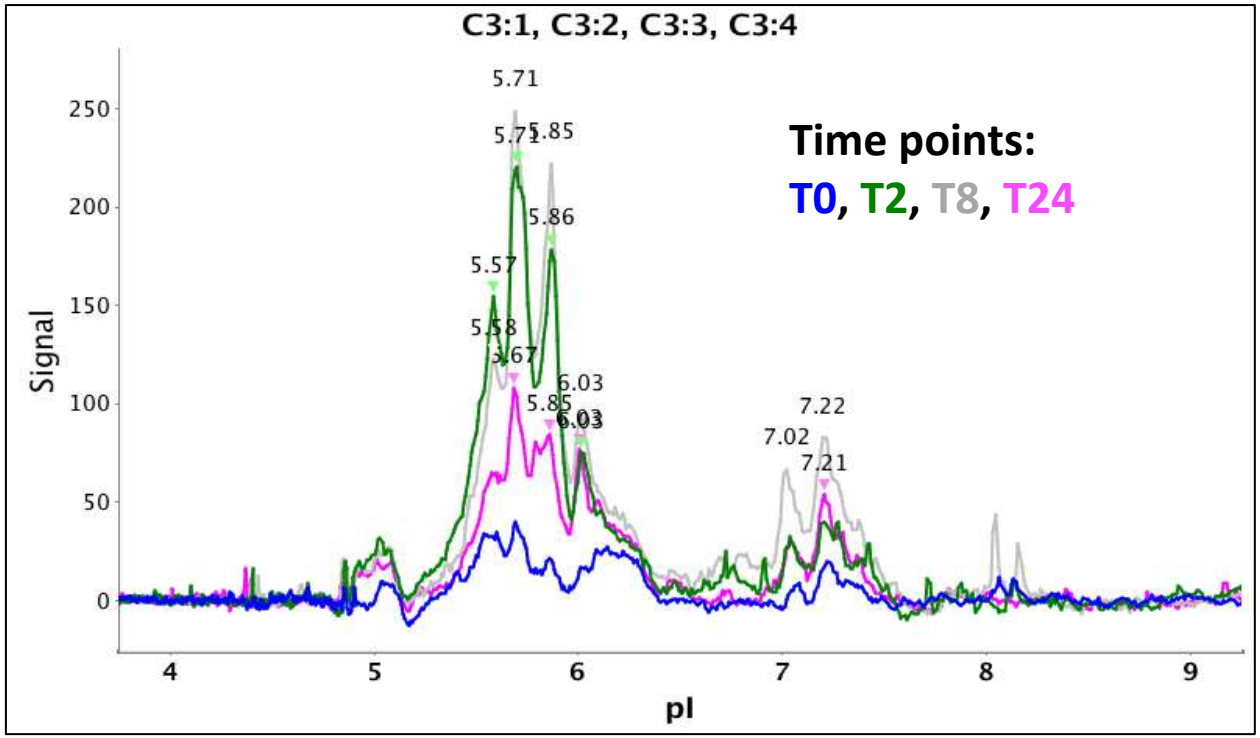


Time course analysis of a drug response in tumor cells extracted by Fine Needle Aspirates.
Drug injected at time 0 and tumor cells analyzed for changes in phosphorylation of proteins examined at time points 2, 8, and 24 hours post injection. Time points 2-24 show significant increases in protein phosphorylation.

Standard Western View



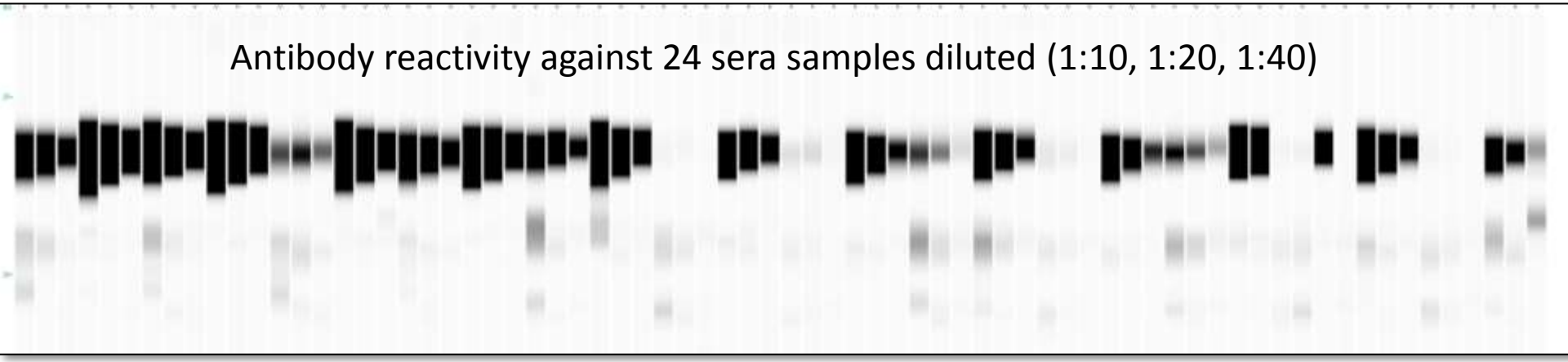
Electropherogram View

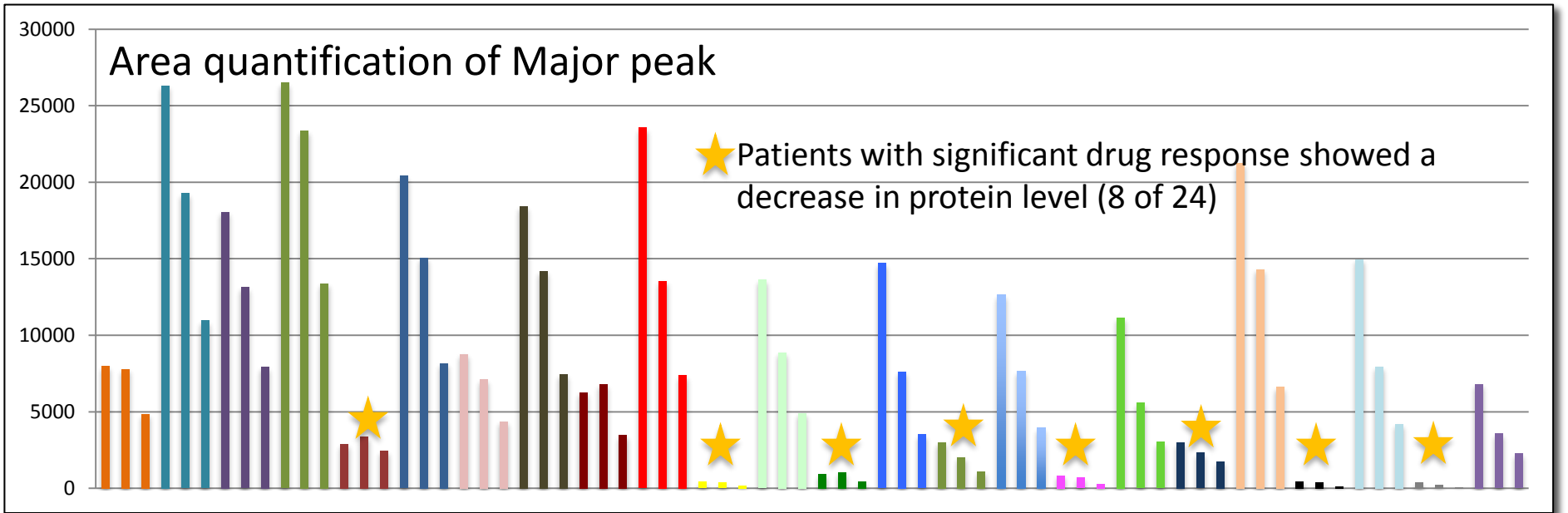


Identification Of Clinical Trial Patient Response

Analysis and Quantification of Proteins from Human Serum Samples

PeggySue can provide accurate, consistent, size measurements of proteins with high-throughput capability.
(up to 96 samples analyzed at once)

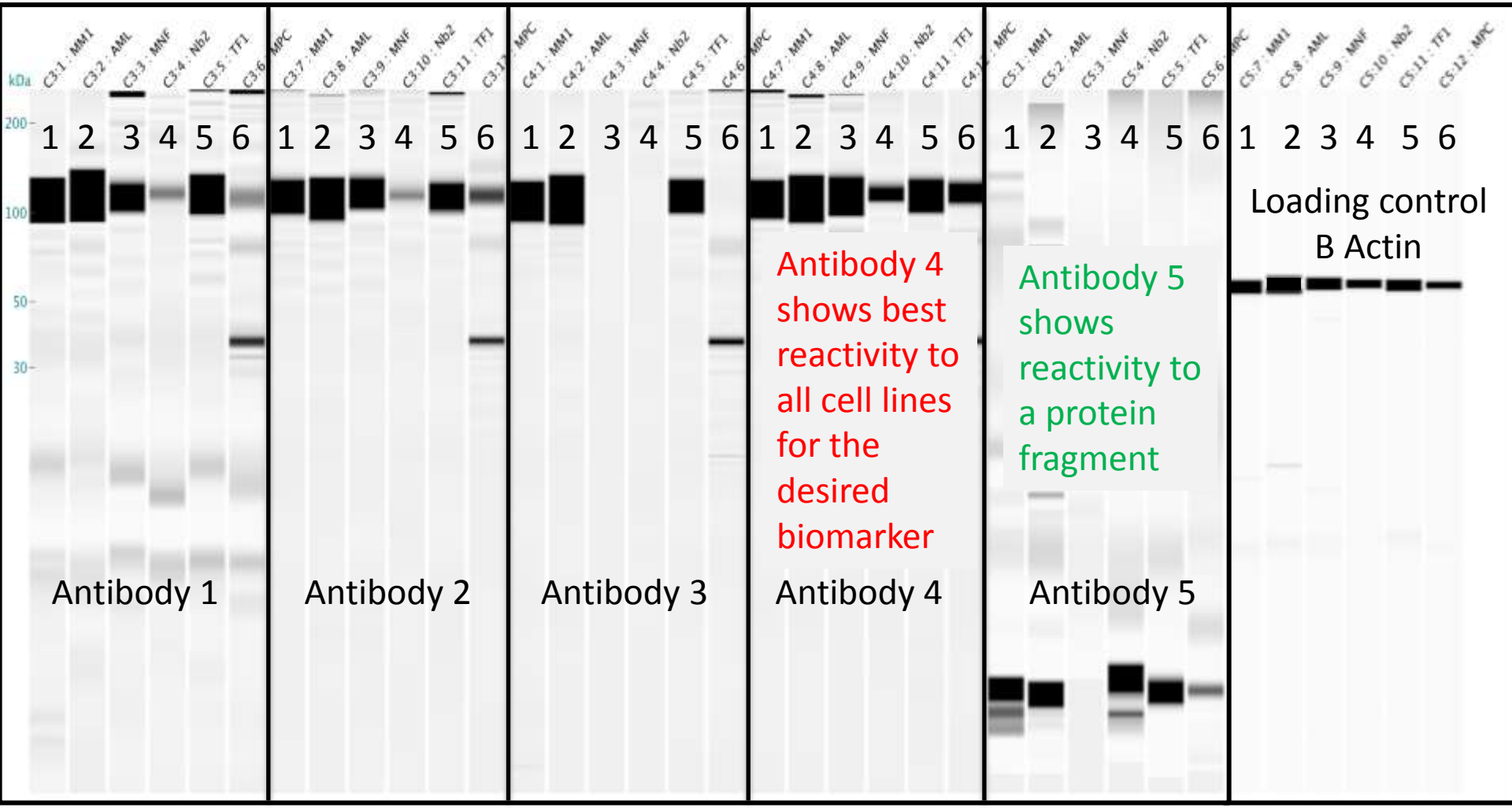




Monoclonal Antibody Analysis
Biomarker Identification

6 different cell lines analyzed simultaneously to determine the best mAb for expression levels of a selected protein.

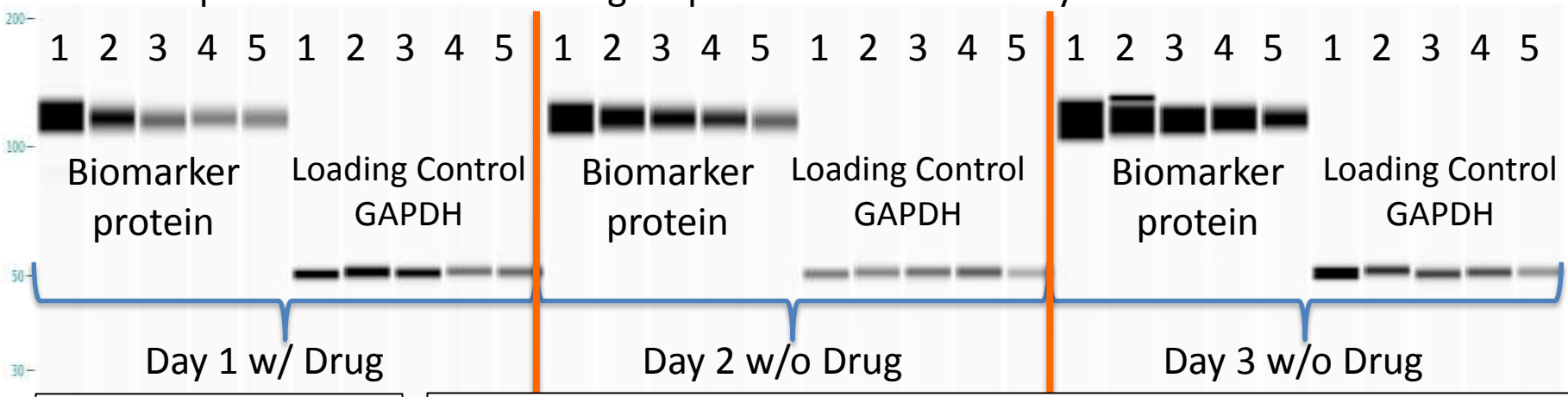
Cell Lines: 1) MM1S, 2) AML5, 3) MNF560, 4) NB2-11, 5) R&D TF1, 6) MPC-11



Drug Response Time Course

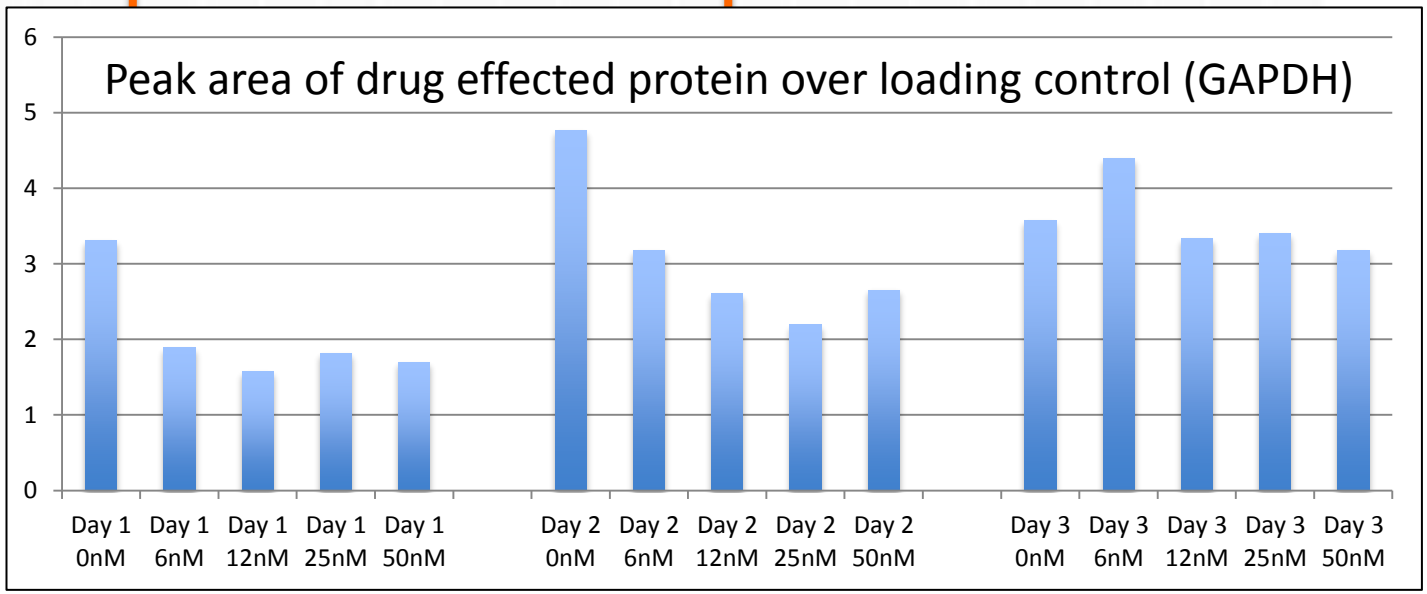
Washout Analysis of Targeted Biomarker

Experiment was conducted to determine the duration of the drug effect. At the initiation of the experiment, drug was added at 4 concentrations. After one day the drug was washed away. Day 1 & 2 show significant decrease of the biomarker protein. Day 3 demonstrated a recovery of the biomarker protein. Conclusion: Drug response lasts for two days.



Drug concentration

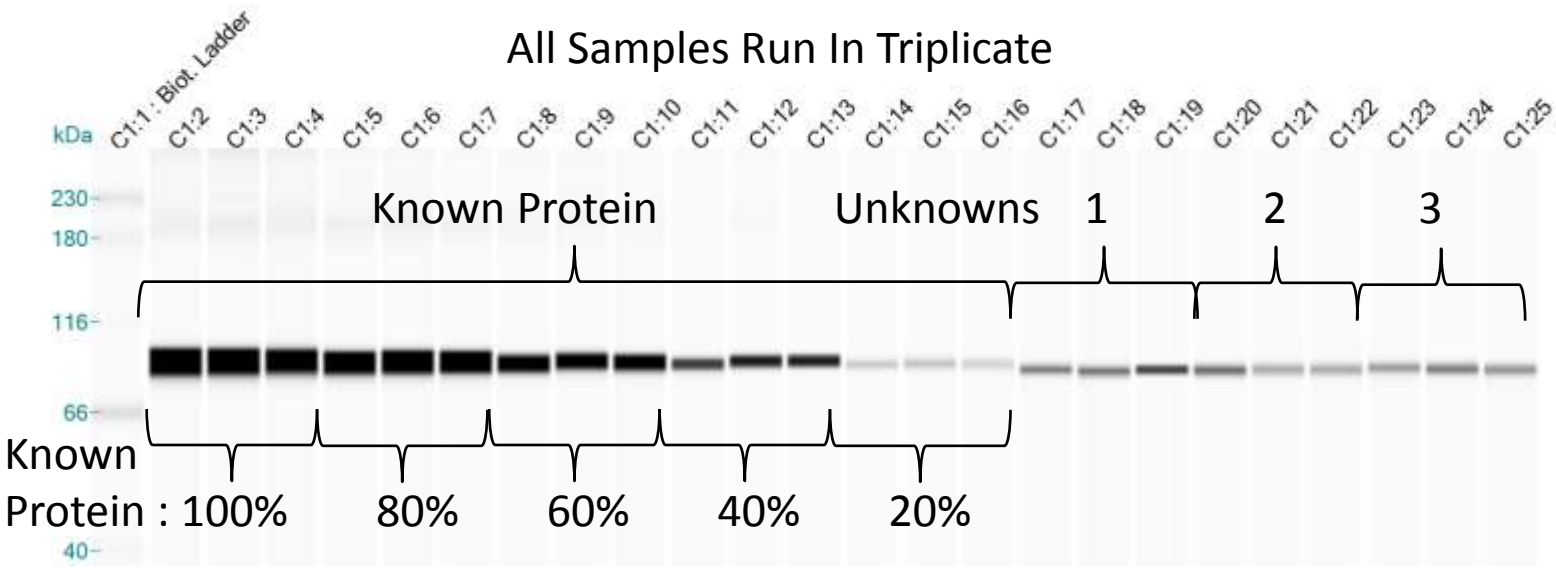
1. 0 nM
2. 6.25 nM
3. 12.5 nM
4. 25 nM
5. 50 nM



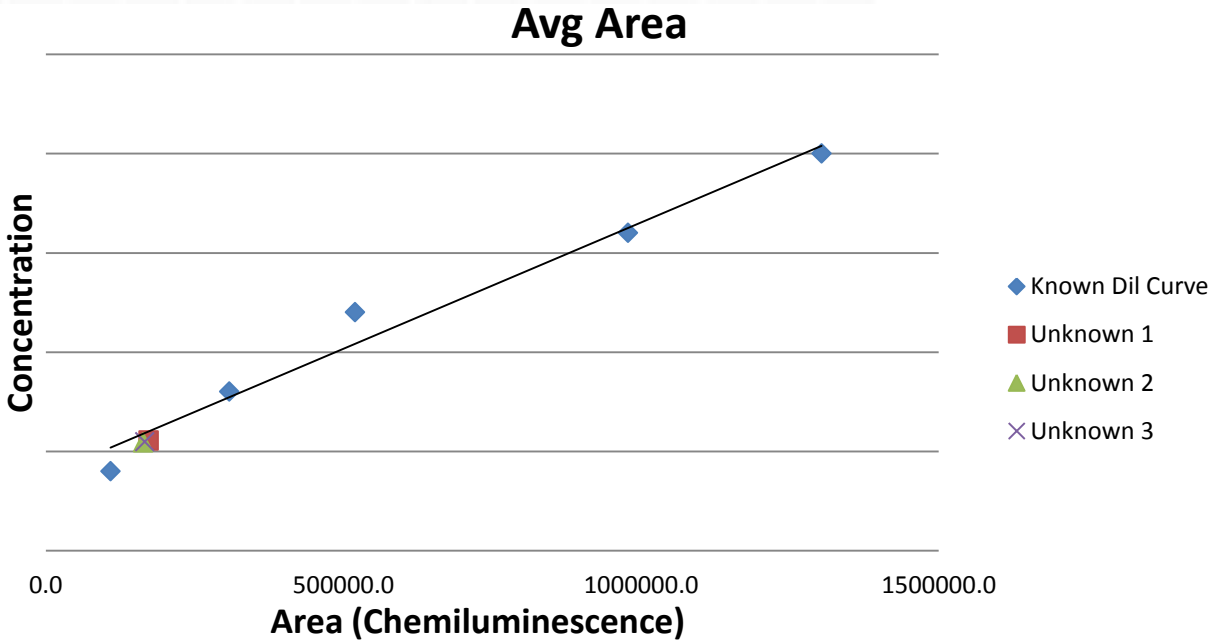
SBH DIAGNOSTICS™

Calculating Concentration of Unknown Biomarker

Using Known Protein Concentration

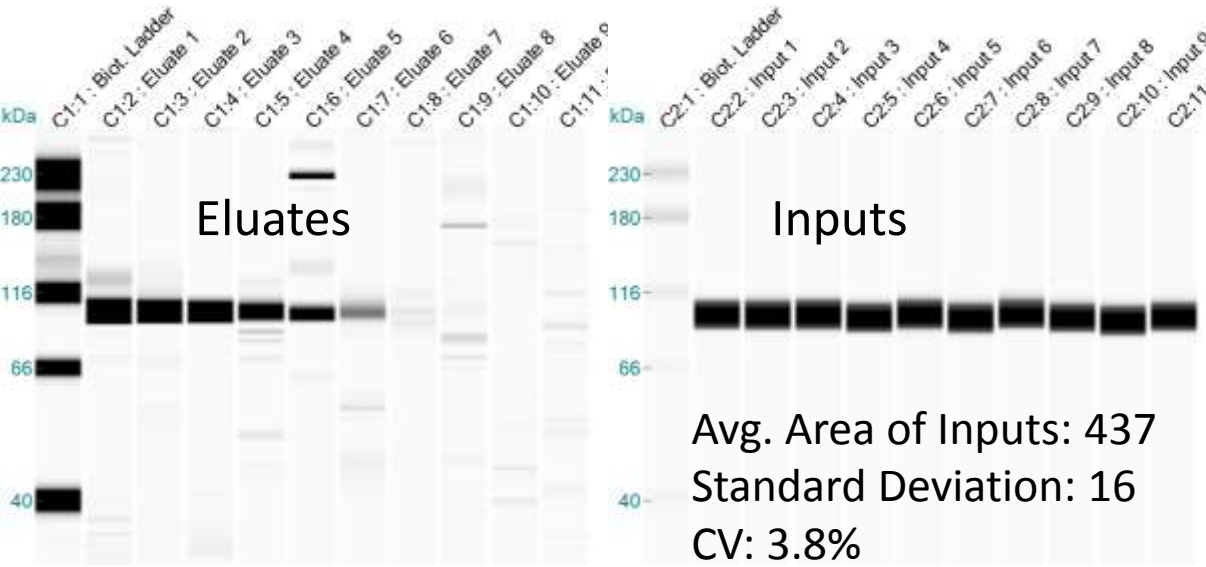


Determining the average of each known protein dilution point allows for statistical calculation of unknown biomarker. Unknown protein concentration is based on the slope of the dilution curve.



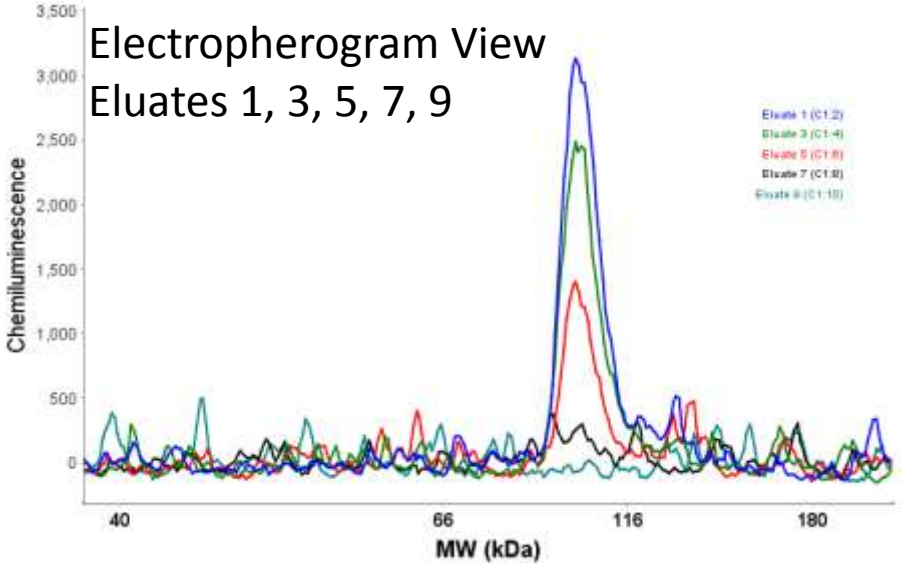
Optimization of Biomarker Recovery

from Clinical Samples size analysis

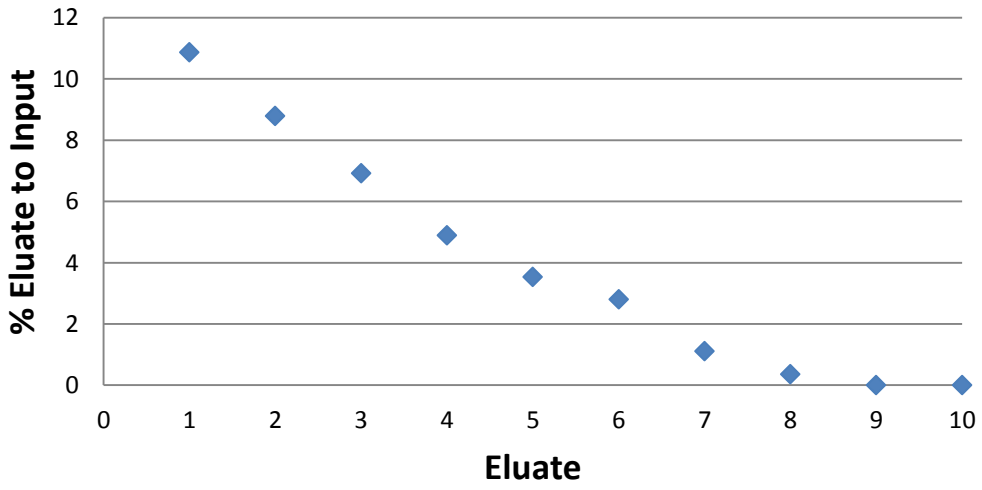


Avg. Area of Inputs: 437
Standard Deviation: 16
CV: 3.8%

Input levels of biomarker are constant. Elution levels of biomarker are reduced every elution for overall full recovery.



Eluate/Input Ratio

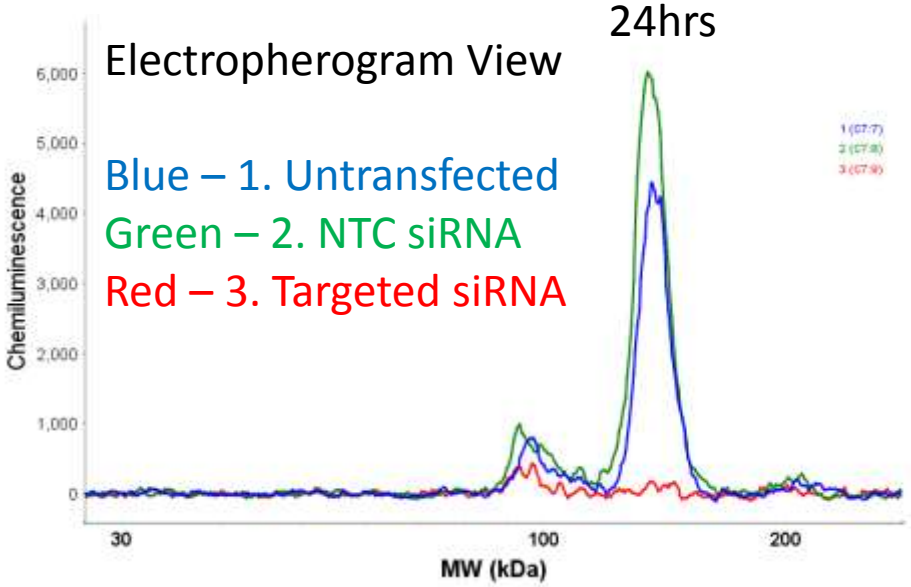


Knockdown Experiment using siRNA

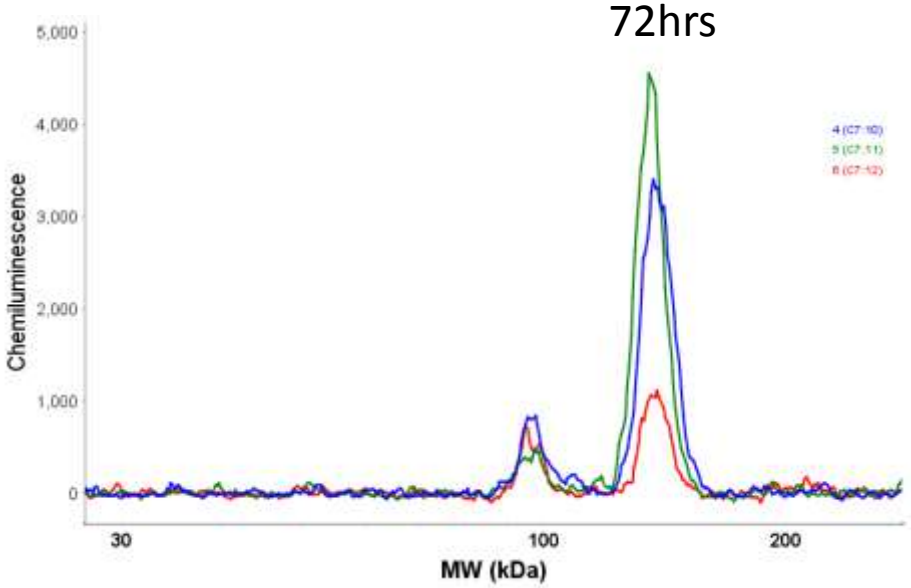
Electropherogram View

24hrs

Blue – 1. Untransfected
Green – 2. NTC siRNA
Red – 3. Targeted siRNA



72hrs



There are three samples at two separate time points. The results clearly demonstrated the knockdown of the targeted protein.

1. Untransfected
2. Non-Targeted Control siRNA
3. Targeted siRNA

Standard Western View