Optimizing a Chip-Based Capillary Electrophoresis Method for Rapid Protein Characterization

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Outline

✓ **Understanding GXII Chip Based Capillary technology**

🔍 **Explore Instrument Parameters**

⚙ **Optimization of Performance for Our Molecules**

▤ **Implementation - Enabling HT Process Development Sample Testing**

▥ **Results and Summary**

🤝 **Conclusion, Lessons Learned, and Future work**

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LabChip GXII Touch - Perkin-Elmer for Capillary Electrophoresis

❑ **A microfluidics-based instrument**

❑ **Applications:**
   ▪ DNA and RNA Analysis: sizing, quantification
   ▪ **Protein Analysis: protein sizing, quantitation, and purity assessments**
   ▪ Genomic Research Automation: Provides high-throughput capabilities

❑ **Data Output:**
   ▪ Generates data **similar to** traditional capillary gel electrophoresis (CE)
   ▪ **Exportable data** into other software: Chromeleon/Empower

❑ **Built in method options based on molecular size and sensitivity options**

❑ **Analysis kits include reagents and chip**

❑ **Under 1 minute per sample using 96 well plate**

Reference: PE LabChip-GX-Touch

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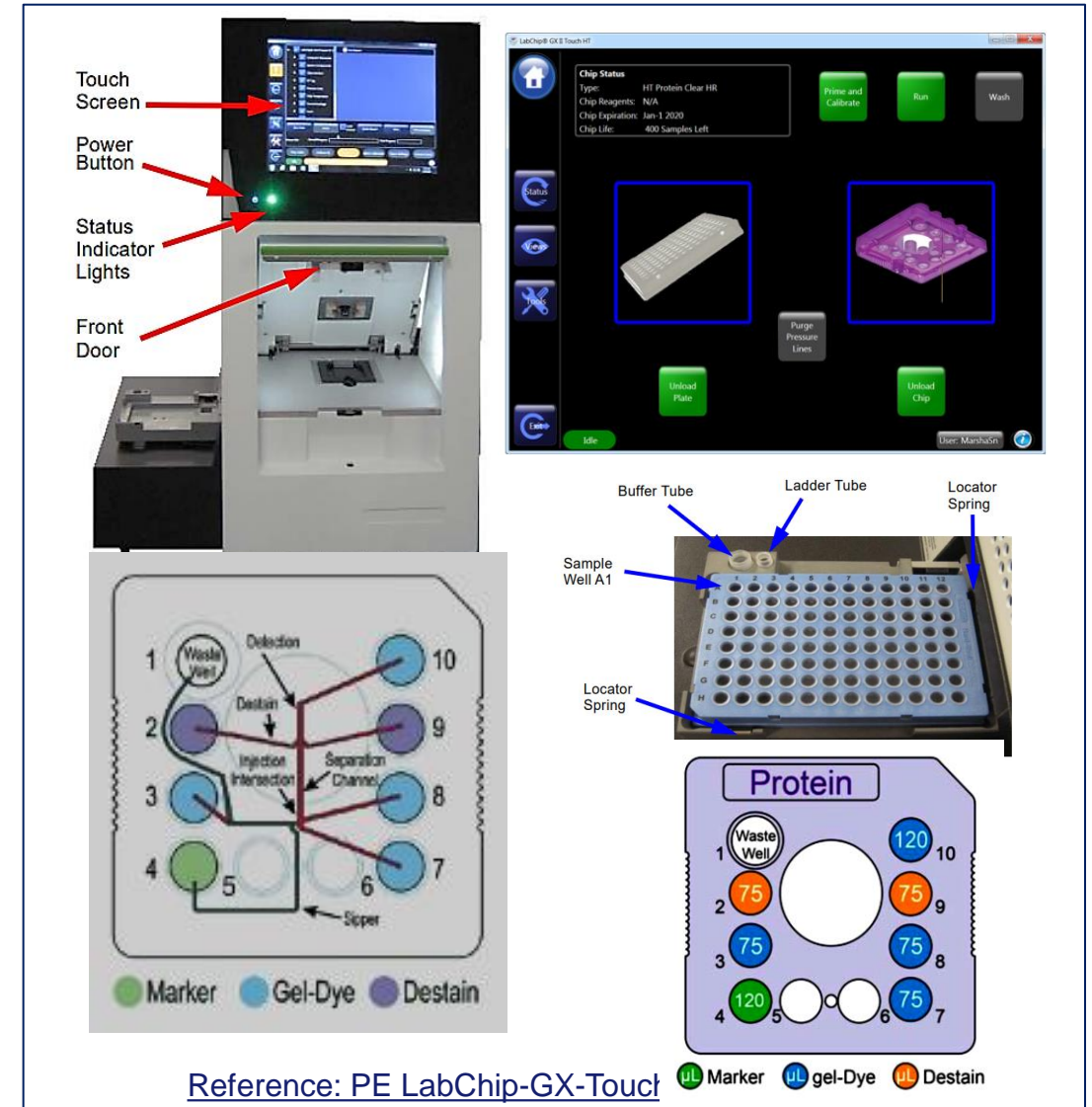
Why is it an ideal system for CE Analysis?

- ❑ **Meet the process development sample testing load**

  - ▪ Sample testing load can reach very high number per week, so utilize the right technology is critical to meet the desired turn-around time.

- ❑ **Fit for purpose Technology use**

  - ▪ Separate the sample by types: Process characterization vs for information only (FIO) technical development
  - ▪ Separate by phase: Early phase clone development selection vs. late phase process parameter decision making

- ❑ **Ready to use method**

  - ▪ Method options included in built-in software to fit different molecule needs
  - ▪ Develop a platform method with quicker optimizations

- ❑ **Time**

  - ▪ Saves time for method development for most new molecules of similar modalities
  - ▪ Under 1 minute analysis for reduced and non-reduced capillary electrophoresis and delivery of up to 96 results during working hours (hands-on and instrument running time: 5 – 6 hours)

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Our Initial Challenges

☐ **Learning and understand the technology**

- Instruments available but not utilized to their full potential to support large sample sets
- Lack of expertise and user knowledge within our lab

☐ **Lack of understanding when problems occur in the data**

- What are the parameter and performance differences of each method option?
- Which method option fits the best to screen our pipeline molecules?
- What are the critical parameters for reagent and sample preparations?

☐ **How to troubleshoot?**

- What to do when observing issues with baseline, sensitivity, and reproducibility?

☐ **Time**

- Support product testing while developing and optimizing the methods

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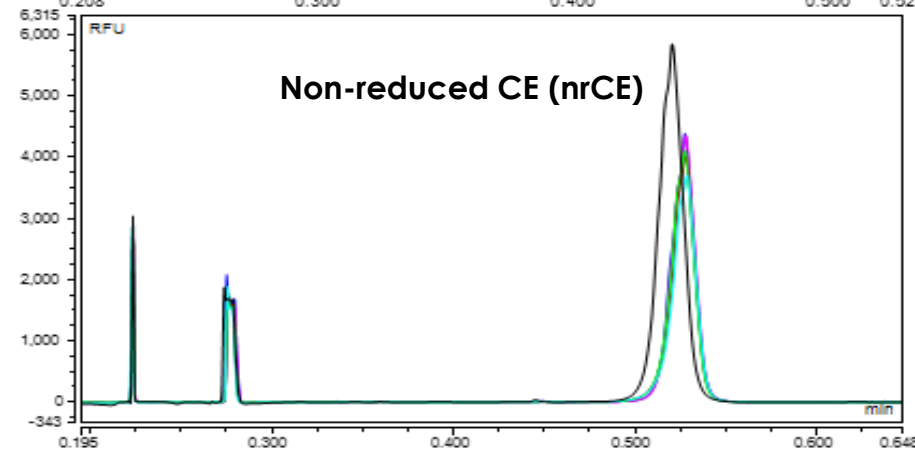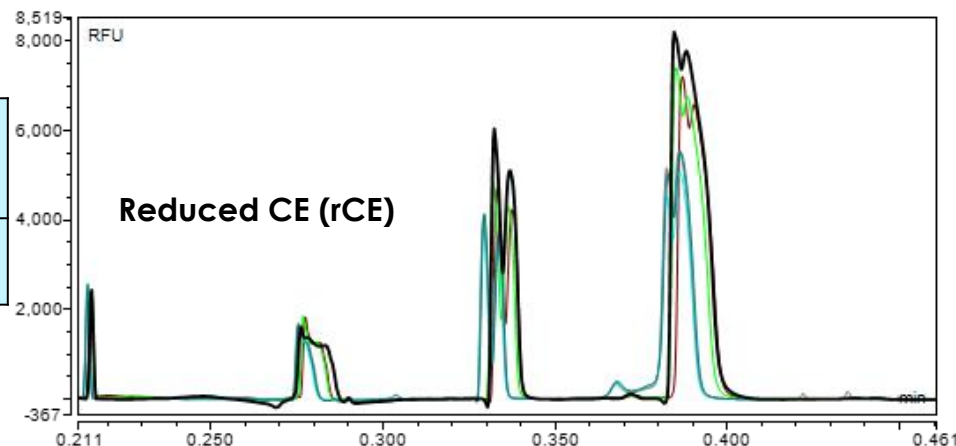# Observed high variabilities during rCE and nrCE analysis

❑ **Method:** HT Protein Express Analysis 200 High Sensitivity , Reagent: follow manufacturer's user manual
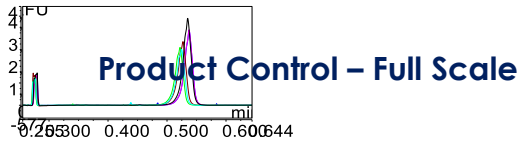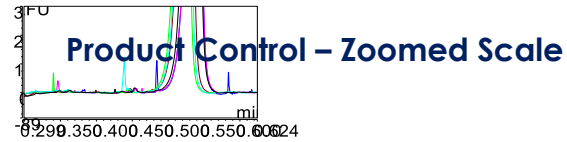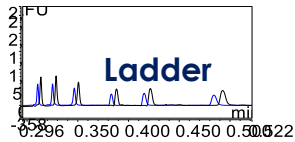
❑ **Profiles**

▪ **Ladder**

Reduced CE (rCE)

Non-reduced CE (nrCE)
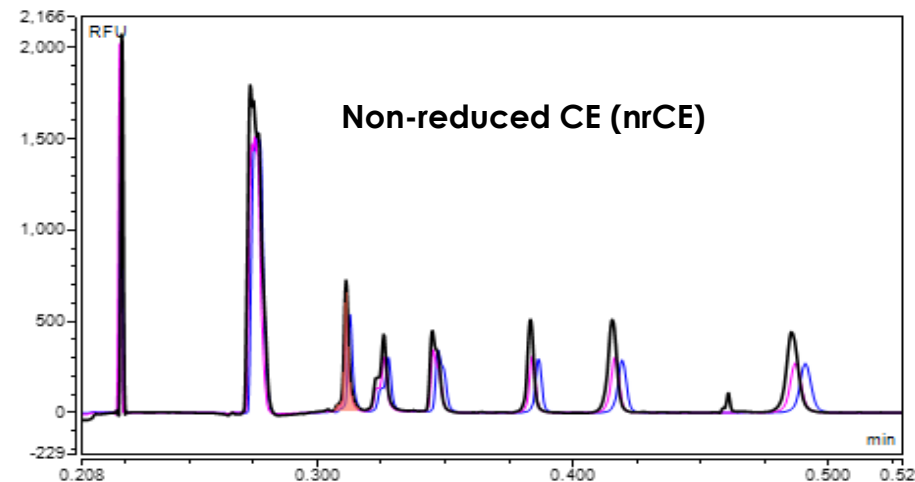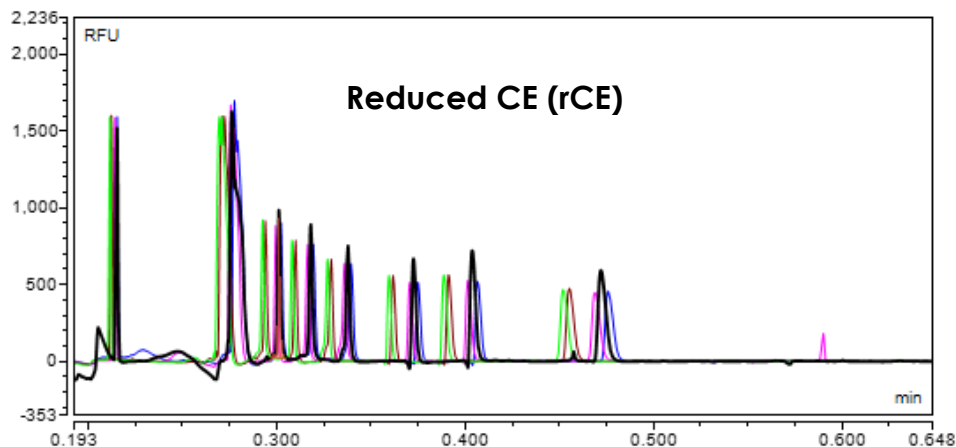
• **Product Control**

	% CA LC1	% CA LC2	% CA Main	Total CA
% RSD	3.1	4.3	0.9	28.0

Reduced CE (rCE)

Non-reduced CE (nrCE)

Highly variable migration time and non-reproducible results!

6

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Observations from nrCE explorative analysis

Ladder

Product Control – Zoomed Scale

Product Control – Full Scale

	Peak Area	Total Corrected Area
	Main Peak (RFU*min)	nV*cm
RS1	65.049	2461478478
RS2	54.475	2052753938
RS3	55.149	2078425245
RS4	48.125	1849739827
RS5	42.897	1664466689
RS6	42.02	1638212706
Average	51.286	1957512814
Standard Deviation	8.7	308906356
%RSD	17.0	15.8

1. **Migration time shifts throughout the run.**
2. **Peak area is not reproducible.**
3. **Non-product related spikes observed randomly in different injections**

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# What are the causes for MT Shift and non-reproducible peak area?

❑ **Review our procedures against manufacturer's "Protein Express Assay User Guide For LabChip® GXII Touch and LabChip® GXII"**

|	Amgen's procedure	Manufacturer's Procedure
Chip	Conform to Manufacturer's Procedure	HT Protein Express LabChip PN 760499
Tubes	Partial conform to Manufacturer's Procedure	Avoid using non-stick lab consumables.
Reducing/Non-Reducing buffer	BME NEM	BME, DTT , or TCEP Literature: IAM
Dye	As recommended by Manufacturer's Procedure with the exception: **Dye spun down before addition to matrix and inverted spin: not mentioned in the procedure**	1. Thaw the dye at room temperature for at least 30 minutes until fully thawed. Vortex the thawed Dye solution for 20 seconds and quickly spin down before use. 2. 520 μL Protein Express Gel Matrix to a spin filter. Add 20 μL Dye solution and spin filter containing the matrix. 3. Cap the filter and vortex in the inverted orientation until the dye is uniform in blue color.
Destain	Conform to spin speed, **shorter spin time**	Spin at 9300 rcp for 8 minutes
Ladder	As recommended by manufacturer's procedure with the exception: **not incubated at 100°C for 5 minutes**	Protein Express Ladder warmed to room temperature, then vortex gently for 10 seconds, briefly spin (centrifuge) . If precipitate visible, sit at room temperature for a longer time, repeat vortex and spin. Add 12 μL to the plate
Sample	Prepared as recommended but incubation per Amgen's procedure. **Add 5 μL sample per 7 μL denaturing buffer**	7 μL denature solution into a tube, pipette 2 μL of each sample (no conc specified, High Protein Express 200 high sensitivity, 5 μL sample for High Protein Express 200). Incubated at 100°C for 5 minutes
Final plate spin	Centrifuge sample at **higher speed** for 5 minutes	Spin the sample plate at 3000 rpm for 5 minutes
Preparing the Chip	As recommended by Manufacturer's Procedure	PerkinElmer's "Protein Express Assay User Guide For LabChip® GXII Touch and LabChip® GXII"
Method	HT Protein Express Analysis 200 High Sensitivity	One option out of 8

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Correct ladder preparation led to reproducible profiles

❑ **Incubate Ladder at 100°C for 5 minutes and samples at 70°C for 10 minutes**

Migration time still shifts from injection to injection, but peak areas are consistent
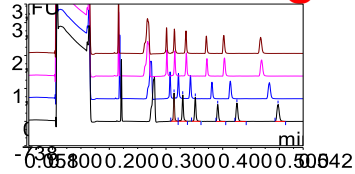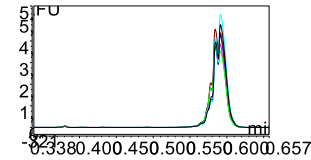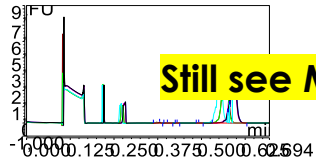

Injection 1
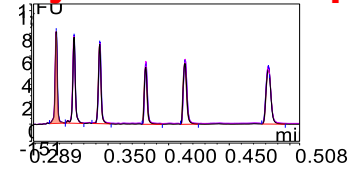
Injection 2

Injection 3

Injection 4

Injection Number (n=4)	RT	RT	RT	RT	RT	RT
	min	min	min	min	min	min
	L1	L2	L3	L4	L5	L6
Average	0.306	0.321	0.342	0.379	0.411	0.479
Standard Deviation	0.0	0.0	0.0	0.0	0.0	0.0
%RSD	2.0	2.2	2.3	2.5	2.6	3.0
≤5.0%						

Injection Number (n=4)	Area	Area	Area	Area	Area	Area
	RFU*min	RFU*min	RFU*min	RFU*min	RFU*min	RFU*min
	L1	L2	L3	L4	L5	L6
Average	1.721	1.881	1.794	1.449	1.864	2.265
Standard Deviation	0.1	0.1	0.2	0.1	0.1	0.1
%RSD	6.7	7.5	8.4	4.4	5.8	5.6
≤10.0%						

Controls from the same nrCE run with improved reproducibility

Still see MT shifts from injection to Injection

Injection Name	Total CA (nV*cm)	Main Peak Mig. Time (min)
RS1	3122577135	0.591
RS2	2831182864	0.593
RS3	2960051268	0.592
RS4	3284124726	0.57
RS5	2731105456	0.57
RS6	3143280384	0.552
Average	3003272141	0.578
Standard Deviation	208714611	0
%RSD	7	3
	≤20.0%	≤5.0%
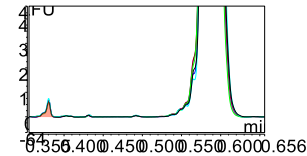
❑ **Migration time shifts throughout the run are acceptable and reported by other users.**
❑ **Possible impact from the dye concentration change in the stain solution per vendor's guide.**

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Different resolution compared to the run on traditional system

Control chromatogram from PA800+ and GXII

nrCE PA800plus
RS Control Full Scale

nrCE GXII
RS Control Full Scale
Integration 1

nrCE GXII
RS Control Zoomed Scale
Integration 1

nrCE PA800plus
RS Control Zoomed Scale

nrCE GXII
RS Control Full Scale
Integration 2
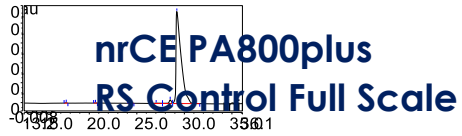
	Injection Name	% CA	% CA	% CA
		Pre-Peaks	Main Peak	Post Peaks
PA800 Plus	RS Control	2.682	97.318	0
GXII Integration 2	RS Control	3.073	96.927	0
GXII Integration 1	RS Control	14.961	85.039	0

- ☐ **Need more understanding on peak/peak group integration using Chip-based method**
- ☐ **Resolution power between 2 methods may not be the same causing more arbitrary data**

# Project demands and timeline needs:

❑ Program A:

- Transfer a non-reduced CE method on GXII from an external lab

- Method performance: inconsistencies in profiles due to unclear sample preparation and instrument procedures at the other lab

- Needs further exploration and optimization efforts

❑ Program B:

- Platform sample preparation procedure run on PA800+ does not work

- Method development on optimizing sample reducing procedure takes time, and process development sample testing is time-sensitive

- Method transfer from method development lab to Rapid Analytics lab: Failed multiple times

- Asked to use GXII to support rCE and nrCE for process characterization samples with minimal notice

- Led to additional efforts to understand and optimize methods

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Gain more understanding through support of Program A

❑ **1st run: Following Manufacture's Procedure HT Protein Express 200 High Sensitivity**
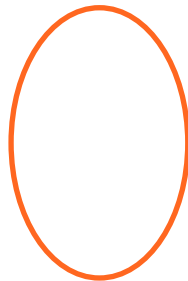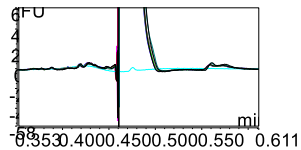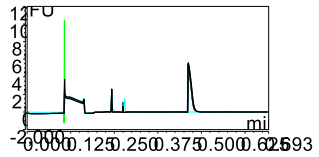


❑ Overlays well
❑ Reproducible
❑ **Problem: Negative spike**

❑ **Reviewed manufacturer's troubleshooting guide and literature:**
  ❑ Protein concentration and denaturing buffer ratio. Recommended 2µL Sample + 7 µL Denaturing buffer when using "High Protein Express 200 High Sensitivity" method option
  ❑ **Experiment plan:**
    ❑ Prepare 5µL Sample + 7 µL Denaturing buffer
    ❑ Prepare 2µL Sample + 7 µL Denaturing buffer

Program A: Change sample to denaturing buffer ratio

☐ **2nd run: Low sample to denature buffer ratio improved: No negative spike was observed!**

High Protein Express 200 High Sensitivity" 5 µL Sample/7µL Denaturing Buffer

High Protein Express 200 High Sensitivity" 2µL Sample/7µL Denaturing Buffer
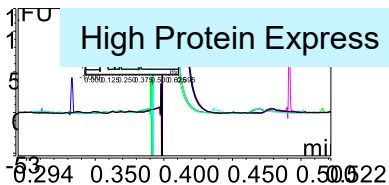
6 replicates at high sample/Denature Buffer ratio
Negative spike present
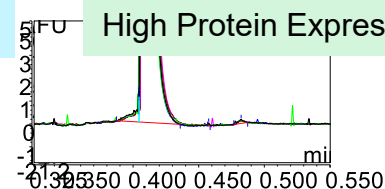
**6 replicates at low sample/Denature Buffer ratio**
No negative spike
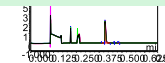
High Protein Express 200 High Sensitivity" 2µL Sample/7µL Denaturing Buffer						
Injection Number (n=6)	% CA	% CA	% CA	Total CA (nV*cm)	Height (RFU)	Mig. Time (min)
	Pre-Peaks	Main Peak	Post Peaks		Main Peak	Main Peak
Average	0.413	99.4765	0.1105	795517894	2790	0.407
Stdev	0.050	0.066	0.045	49496896	138	0.001
%RSD	12.1	0.1	40.7	6	5	0.2

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Lower sample/denature buffer ratio showed better profiles

❑ **3 runs are compared on 2 different instruments at 2 μL sample /7 μL Denaturing Buffer**

- ▪ **No negative spike!**

- ▪ **Wavy and raised baselines:**
  - o Dirty electrode: Most likely. The same sample was analyzed
  - o High detergent concentration in sample: unknown for this sample

- ▪ **Different response: Peak heights on 2 different instruments.**
  - o HT Protein Express Analysis 200 vs HT Protein Express Analysis 200 High Sensitivity Method
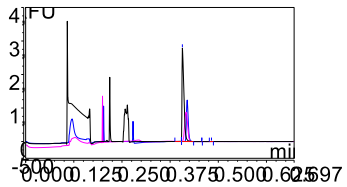
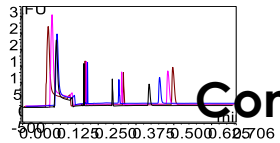**HT Protein Express Analysis 200 high Sensitivity**

**HT Protein Express Analysis 200**

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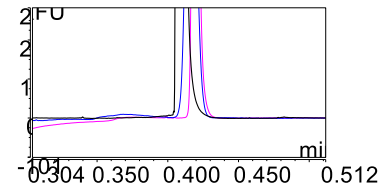
Sample concentration range acceptable for Program A

❑ **Different sample concentrations at 2 µL sample/7 µL Denaturing Buffer Using "HT Protein Express 200" option**
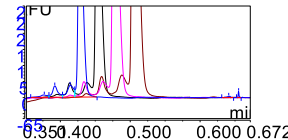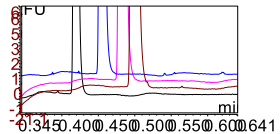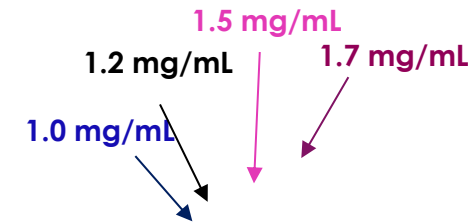
- No negative spike!
- Similar profiles in control and stressed samples between 1.0 – 1.7 mg/mL

Control

1.5 mg/mL

1.2 mg/mL

1.7 mg/mL

1.0 mg/mL

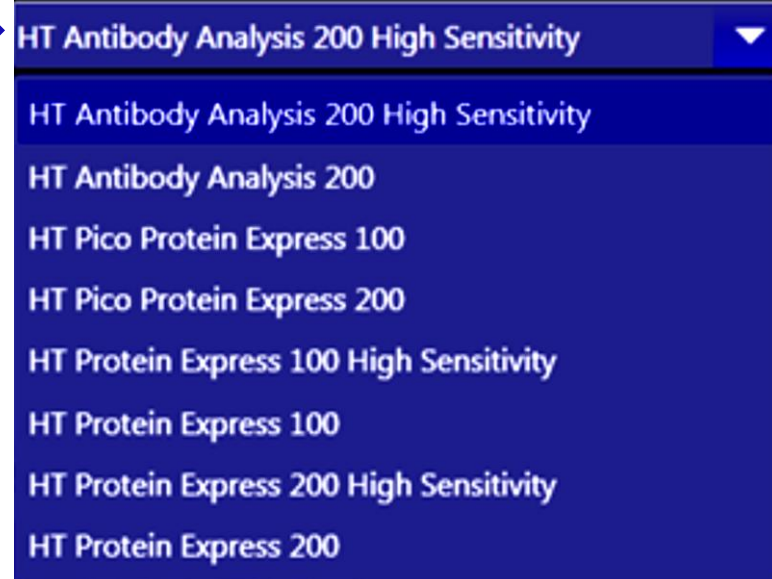**3 months 40C**

1.5 mg/mL

1.2 mg/mL

1.7 mg/mL

1.0 mg/mL
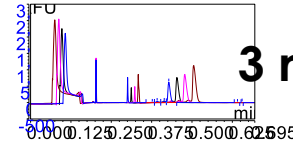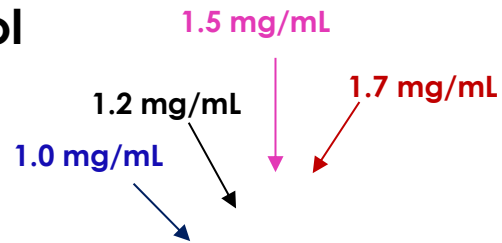
Supporting Program B with what we have learned from Program A

Program A:

❑ Method Option: HT Protein Express 200

❑ Non-Reduced CE-SDS: Sample Concentration: 1.0 – 1.7 mg/mL

❑ Sample Volume to Denaturing Buffer Ratio: 2µL /7µL

Program B

❑ Support process development sample testing using the above parameters

❑ Explore other method options: ⟶

❑ Explore rCE-SDS testing using similar strategies

| HT Antibody Analysis 200 High Sensitivity ▼ |
| --- |
| HT Antibody Analysis 200 High Sensitivity |
| HT Antibody Analysis 200 |
| HT Pico Protein Express 100 |
| HT Pico Protein Express 200 |
| HT Protein Express 100 High Sensitivity |
| HT Protein Express 100 |
| HT Protein Express 200 High Sensitivity |
| HT Protein Express 200 |

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Program B: First round

- Method Option: HT Protein Express 200
- Non-Reduced CE-SDS: Sample Concentration: 1.07 mg/mL
- Sample Volume to Denaturing Buffer Ratio: 2µL /7µL
- 3 Ladder before and after 20 samples
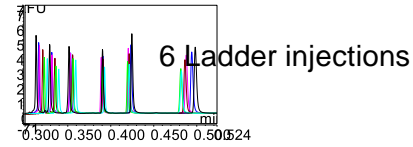
Sample#	% CA Pre-Peaks	% CA Main Peak	% CA Post Peaks
1	15.462	84.380	0.158
2	1.541	98.317	0.142
3	1.380	98.450	0.169
4	1.508	98.492	0.000
5	1.334	98.666	0.000
6	1.570	98.254	0.176
7	1.276	98.566	0.158
8	1.932	97.962	0.106
9	2.034	97.795	0.171
10	2.335	96.828	0.837
11	2.271	97.031	0.699
12	2.404	96.666	0.930
13	1.727	98.090	0.183
14	1.815	97.997	0.188
15	1.803	98.041	0.157
16	1.951	97.273	0.776
17	2.038	97.327	0.635
18	2.050	97.352	0.598
19	1.965	97.322	0.713
20	2.528	96.725	0.747
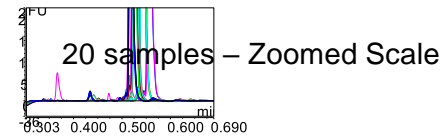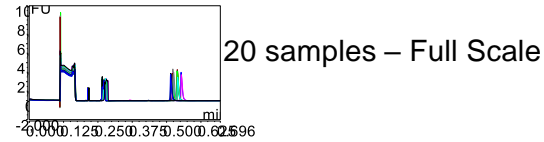
- **Acceptable performance and results are achieved for ladders and samples.**

6 Ladder injections

20 samples – Full Scale

20 samples – Zoomed Scale

Program B run with 21 Samples – successfully completed
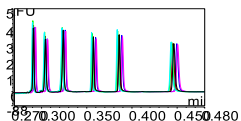
6 Ladder Injections
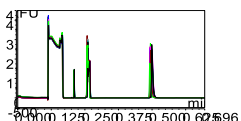
21 samples – Full Scale

21 samples –
Zoomed Scale
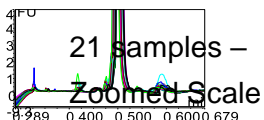
Sample #	% CA Pre-Peaks	% CA Main Peak	% CA Post-Peaks
1	0.889	98.993	0.118
2	2.096	97.405	0.500
3	1.132	98.404	0.464
4	1.637	97.918	0.446
5	2.065	97.470	0.465
6	1.737	97.707	0.557
7	1.929	97.024	1.048
8	1.036	98.608	0.356
9	1.144	98.353	0.503
10	1.194	98.619	0.187
11	1.312	98.078	0.610
12	1.711	97.899	0.390
13	0.898	98.515	0.588
14	0.779	97.828	1.393
15	1.204	98.179	0.617
16	1.129	98.335	0.535
17	1.017	98.367	0.616
18	1.508	97.828	0.665
19	1.066	98.603	0.332
20	1.035	98.556	0.408
21	1.503	97.924	0.573

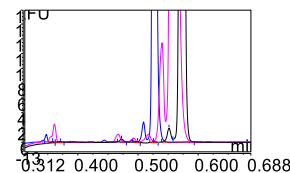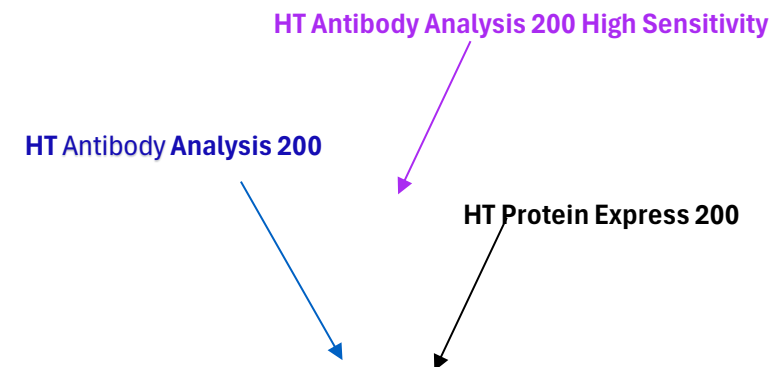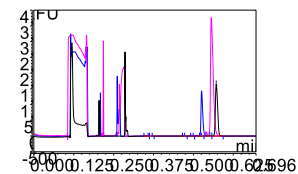❑ **Another 2 subsequent sample sets for a total of 65 samples were analyzed successfully!**

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Explore different nrCE method options using Program B molecule

Understanding method options

HT Antibody Analysis 200 High Sensitivity ▼

HT Antibody Analysis 200 High Sensitivity
HT Antibody Analysis 200
HT Pico Protein Express 100
HT Pico Protein Express 200
HT Protein Express 100 High Sensitivity
HT Protein Express 100
HT Protein Express 200 High Sensitivity
HT Protein Express 200
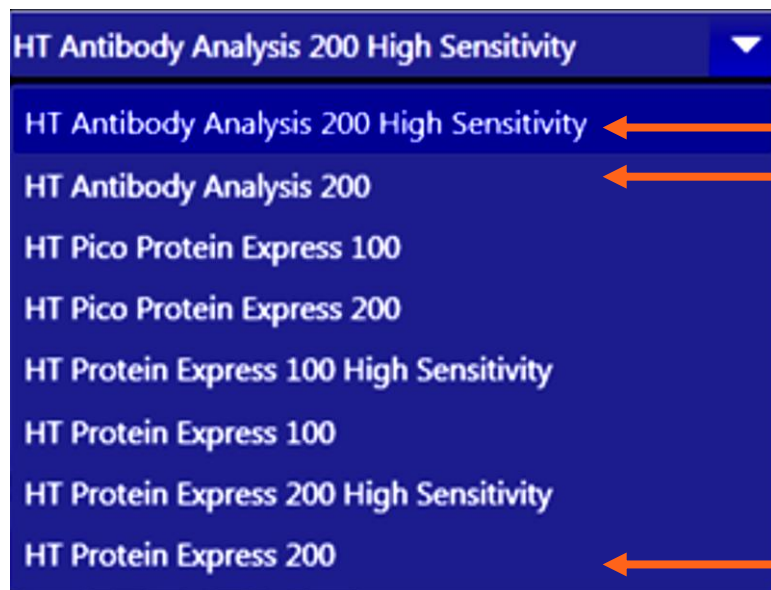
Next step options to explore

Method used for past runs

HT Antibody Analysis 200 High Sensitivity

HT Antibody Analysis 200

HT Protein Express 200

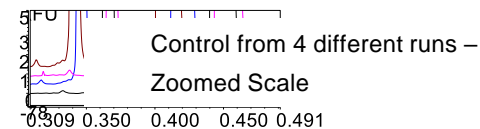| Method	% CA Pre-Peaks	% CA Main Peak	% CA Post-Peaks
HT Protein Express 200	1.691	98.309	0.000
HT Antibody Analysis 200	3.469	96.531	0.000
HT Antibody 200 High Sensitivity*	4.431	95.569	0.000

*Considered an optimized method option

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# Concurrent testing for rCE-SDS analysis at non-optimized conditions

Program B

❑ Following a non-optimized internal procedure using "HT Protein Express 200 High Sensitivity" method option for rCE-SDS:

▪ Incubation: samples at 70°C for 20 minutes per molecule's need.

▪ Ladders at 70°C for 5 minutes vs recommended: 100°C for 5 minutes for both samples and ladders

❑ Supported the same set of samples for nrCE-SDS for 106 samples in total, 5 sets of runs.

Ladders from 4 different runs

Control from 4 different runs – Full Scale

Control from 4 different runs – Zoomed Scale
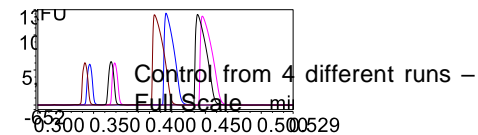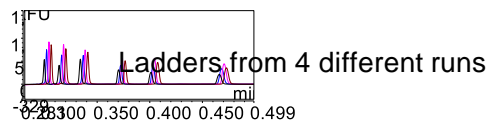
❑ **Both ladders and controls showed consistent profiles.**
❑ **Overloading is observed**

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Program B rCE sample profiles from first four runs

20 samples – Run #1

21 samples – Run #2

20 samples – Run #3

21 samples – Run #4
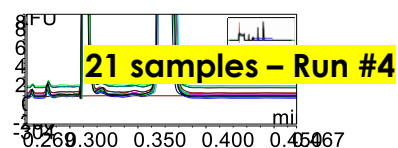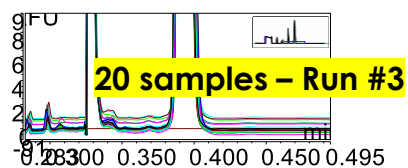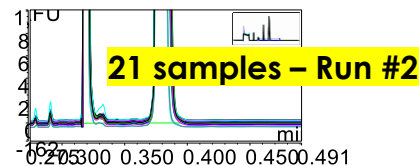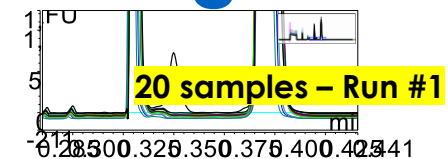
❑ **Acceptable profiles to provide FIO information to the program team**
❑ **A small negative spike is observed before LC peak in all sample injections: Sample overloading based on experience gained from nrCE. RFU response is > 1000 RFU as recommended.**

# Explore different instrument method options for rCE-SDS

Understanding method options

HT Antibody Analysis 200 High Sensitivity

- HT Antibody Analysis 200 High Sensitivity ← **Next step options to explore**
- HT Antibody Analysis 200 ←
- HT Pico Protein Express 100
- HT Pico Protein Express 200
- HT Protein Express 100 High Sensitivity
- HT Protein Express 100
- HT Protein Express 200 High Sensitivity ← **Method used for past runs**
- HT Protein Express 200 ←

HT Antibody Analysis 200 High Sensitivity

HT Antibody Analysis 200

HT Protein Express 200 High Sensitivity

HT Protein Express 200
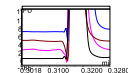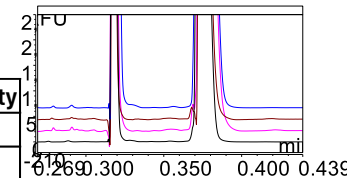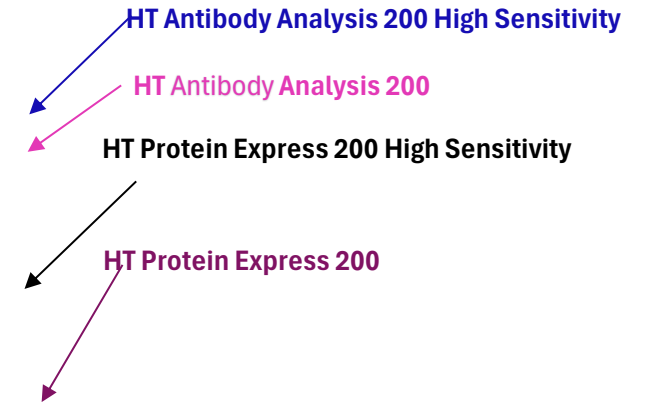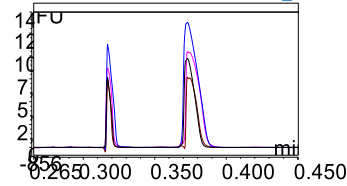
| Injection Name	% CA LMW	% CA LC	% CA MMW	% CA NGHC	% CA HC	% CA HMW	% CA Clips	% CA Purity
Sample A, HT Protein Express 200 High Sensitivity	0.295	28.259	0.194	0.676	70.504	0.073	0.489	98.763
Sample A, HT Protein Express 200	0.424	30.913	0.747	0.164	67.683	0.070	1.170	98.597
Sample A, HT Antibody Analysis High Sensitivity	0.161	28.381	0.195	0.067	71.185	0.011	0.356	99.566
Sample A, HT Antibody Analysis	0.165	28.538	0.222	0.032	71.035	0.007	0.388	99.573

Observations:

1. Negative spike before LC peak is observed under all conditions
2. RFU response is > 1000 with 5/7 sample to denaturing buffer ratio

Further explore ratios of sample to denaturing buffer

- ❑ Reducing Agent BME vs DTT with Sample/Denaturing Buffer Ratio: 1µL-7µL sample/7 µL Denaturing Buffer
- ❑ Incubation: Sample at 70C for 20 minutes, Ladders at 70C for 5 minutes
- ❑ HT Protein Express Analysis 200 High Sensitivity

rCE using BME
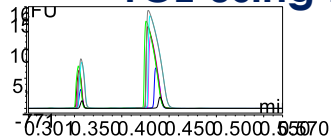
rCE using DTT

Sample: 1µL to 7 µL
Denaturing Buffer: 7 µL
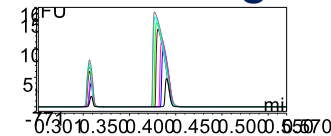
Sample: 1µL to 7 µL
Denaturing Buffer: 7 µL

rCE using BME Zoomed Scale
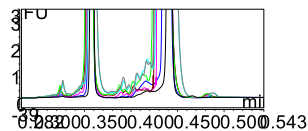
rCE using DTT Zoomed Scale

- ❑ **Overloading was observed for BME with sample volume from 3 µL to 7 µL**
- ❑ **Overloading was observed for DTT with sample volume from 2 µL to 7 µL with a negative spike**

24

BME and DTT results comparison

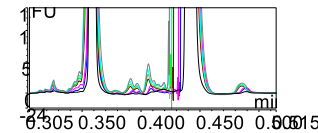| Reducing Agent: BME	% CA LMW	% CA LC	% CA MMW	% CA HC	% CA HMW
1 µL Sample	0.2	37.1	2.5	59.9	0.3
2 µL Sample	0.3	27.0	1.8	70.8	0.1
3 µL Sample	0.3	21.9	0.6	77.2	0.1
4 µL Sample	0.3	21.4	0.5	77.8	0.1
5 µL Sample	0.3	21.2	0.3	78.1	0.1
6 µL Sample	0.3	21.0	0.5	78.1	0.1
7 µL Sample	0.4	20.7	0.5	78.4	0.1
Reducing Agent: DTT	% CA LMW	% CA LC	% CA MMW	% CA HC	% CA HMW
1 µL Sample	0.1	25.1	0.5	74.2	0.0
2 µL Sample	0.2	22.2	0.2	77.3	0.0
3 µL Sample	0.3	22.1	0.2	77.4	0.1
4 µL Sample	0.2	21.2	0.2	78.4	0.1
5 µL Sample	0.1	21.0	0.2	78.6	0.1
6 µL Sample	0.1	20.7	0.2	78.9	0.1
7 µL Sample	0.2	20.4	0.2	79.1	0.1
Reducing Agent: BME	% CA LMW	% CA LC	% CA MMW	% CA HC	% CA HMW
Control Data from PA800plus	0.0	25.4	0.6	72.3	1.7

2 µL Sample/7 µL BME reducing buffer

1 µL Sample/7 µL DTT reducing buffer

1 µL Sample/7 µL BME reducing buffer

2 µL Sample/ 7 µL BME reducing buffer

1 µL Sample/ 7 µL DTT reducing buffer

1 µL Sample/ 7 µL BME reducing buffer

- ❑ 1 µL to 2 µL sample using BME and 1 µL sample with DTT provided acceptable profiles.
- ❑ Higher % MMW in the BME reduced samples were observed mostly due to the control was incubated 70ºC for 20 minutes vs the Control data incubated at 70ºC for 10 minutes.
- ❑ For future molecules, the incubation time and temperature should be matched to the traditional methods
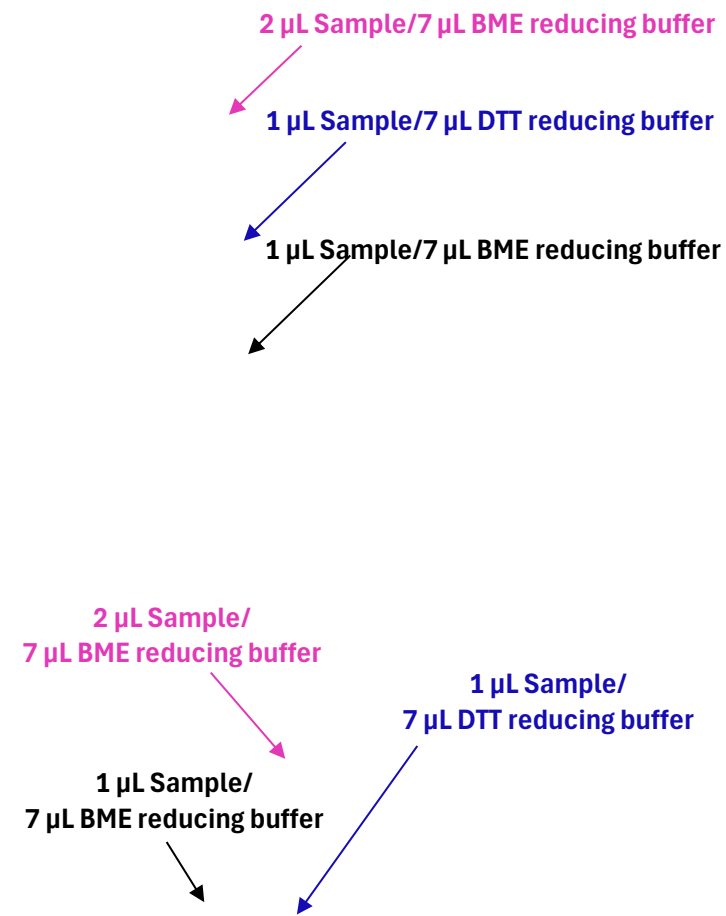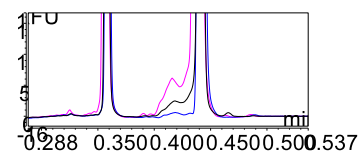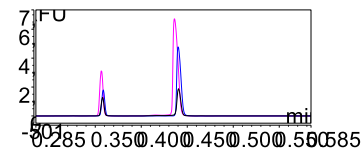
Lessons learned and conclusions

❑ **Critical Steps Impacting Method & Instrument Performance**

- Reagent/Sample Preparations: Accurate preparation of dye solutions, stain solutions, samples, and ladders.
- Stain Solution Homogeneity: Ensure uniformity to minimize migration time shifts.
- Sample to Denaturing Buffer Ratio: Optimize for each molecule for the best profile and sensitivity.
- Cleaning Protocol: Regularly clean chips and plates prior to loading reagents and samples.
- Particulate Removal: Filter samples to remove particulates, preventing blockages.

❑ **Instrument Optimization**

- Method Selection: Choose optimal method options from the instrument manual.
- Water Injection Cleaning: Implement between sample injections to reduce artifact peaks.
- Training Importance: Proper training is essential for reproducibility and troubleshooting.

❑ **Successful Optimization:**

- Both nrCE and rCE LabChip methods were optimized to support early development samples.
- Achieved analysis at 1 minute per sample, up to 96 samples per run, with a total run time of <2 hours.

❑ **Ladder Utility:**

- Provided size information for peaks, aiding in molecule characterization and process optimization.

❑ **Knowledge Gained:**

- Understanding of LabChip GXII for CE analysis was enhanced.
- Proper training is crucial due to the system's complexity.

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Future work

❑ Further explore reducing/non-reducing reagent performance against our platform reagents, e.g., Dithiothreitol (DTT), Tris(2-carboxyethyl)phosphine (TCEP) vs. β-Mercaptoethanol (BME), and Iodoacetamide (IAM) vs. N-Ethylmaleimide (NEM).

❑ Continue to gain understanding of the performance differences among different instrument method options, different reducing agents, and different instrument types.

❑ Acquire expertise for this user-friendly yet complex system to ensure successful operation through well-established training procedures and accumulating troubleshooting skills.

❑ Investigate applications of other instrument method options for future pipeline molecule testing needs such as Pico Protein Express for low concentration protein formulations and fast glycan analysis.

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References

1. LabChip GX Touch/GX Touch II User Manual

2. Characterization of NISTmAb with Microfluidic CE-SDS using the Protein Clear™ HR Assay

Acknowledgments

Amgen Attribute Sciences

David Semin
Margaret Ricci
Amgen Thousand Oaks Rapid Analytics Team

Amgen Legal

Scott Siera

AMGEN

Backup

LabChip GXII Touch - Perkin-Elmer for Capillary Electrophoresis

- ❑ **A microfluidics-based instrument** that utilizes microfluidic chips to automate and **simplify electrophoresis**, providing high-resolution separation and analysis of biomolecules.
- ❑ **Applications:**
  - ▪ DNA and RNA Analysis: sizing, quantification, and quality assessment of DNA and RNA samples, including fragments, smears, and ladder standards.
  - ▪ **Protein Analysis: protein sizing, quantitation, and purity assessments**
  - ▪ Genomic Research: various genomic applications including next-generation sequencing (NGS) sample preparation and quality control.
- ❑ **Automation:** Provides **high-throughput** capabilities and increases efficiency and consistency.
- ❑ **Data Output:**
  - ▪ Generates electropherogram data **similar to** traditional capillary gel electrophoresis (CE)
  - ▪ **Exportable data** into other chromatographic data processing software: Chromeleon/Empower
- ❑ **Built in Instrument Method Options:** Different molecular size range and sensitivity method options
- ❑ **Analysis Kits:** Reagents and Chip are available in 1 kit
- ❑ **Fast:** Under 1 minute per sample using 96 well plate that enables speed in process optimization and decision-making processes

Reference: PE LabChip-GX-Touch