# High throughput CE-SDS protein analysis using fully automated sample and reagent preparation workflow

## Introduction

Therapeutic biologics have revolutionized modern medicine and now represent a multi-billion-dollar business, with hundreds currently approved by the FDA and other international regulatory agencies and thousands more in the development pipeline. These molecules range from traditional monoclonal antibodies, antibody-drug conjugates, multi-specific antibodies, fusion proteins, nanobodies and peptibodies to cell and gene therapy products such as lipid nanoparticles and adeno-associated viruses. In the fast-paced environment of upstream bioprocessing of therapeutic biologics, many samples must be prepared and analyzed to determine and quantify the purity and heterogeneity by chromatographic or electrophoretic means. Such a high number of samples tends to quickly become a bottleneck. Therefore, to address the throughput limitation in the analytical characterization of intermediate samples, this application note demonstrates the fully automated preparation of reduced monoclonal antibodies using the BioPhase CE-SDS Protein Analysis kit and the Biomek i5 MC liquid handler, with downstream analysis using the BioPhase 8800 system. This method also allows for preparing all reagent plates used for the electrophoretic separation by CE-SDS.

The BioPhase CE-SDS Protein Analysis kit is used to resolve reduced and non-reduced proteins by size and quantify the heterogeneity and impurities that might exist in a therapeutic monoclonal antibody.

The methodology involves heat denaturation of protein in the presence of sodium dodecyl sulfate (SDS) and β-mercaptoethanol as reducing agent for reduced samples. The alkylating agent iodoacetamide may be used in case non-reduced samples are desired. Once denatured, the sample is separated by hydrodynamic size in a capillary containing a replaceable SDS polymer matrix, which provides the sieving selectivity for the separation.

The automated method for the BioPhase CE-SDS Protein Analysis kit on the Biomek i5 MC liquid handler can prep from 8 to 96 samples in increments of 8. The user can select the preparation of samples and sample inlet tray, reagent inlet and outlet trays and the sample outlet tray. The sample outlet, reagent outlet and inlet tray prep sections can be operated from start to finish with a single user interface to keep labware and reagents on deck. For the sample prep portion, the liquid handler is paused so the user can add a sealing film on the processing plate before the robotic arm transfers the plate to the Peltier for heating. In this application note, we will demonstrate the performance of the BioPhase CE-SDS Protein Analysis kit on the Biomek i5 MC Workstation. The BioPhase CE-SDS Protein Analysis kit on the Biomek i5 MC automated method provides:

- Reduced hands-on time
- Reduced potential for pipetting errors
- Quick installation with ready-to-implement method

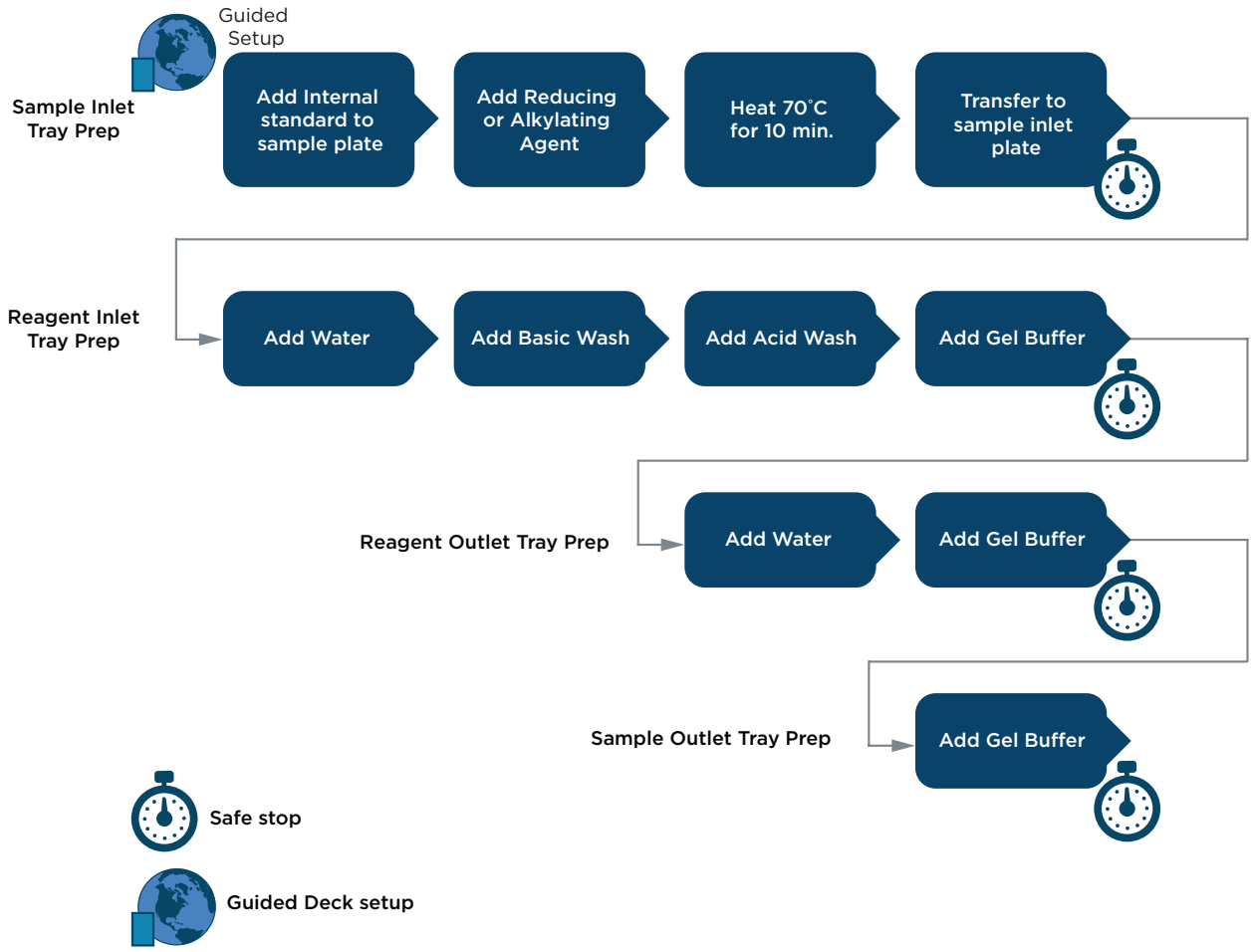


**Figure 1:** Workflow of automated method for the BioPhase CE-SDS Protein Analysis kit on the Biomek i5 MC Workstation.

## Spotlight

The Biomek i5 MC Automated Workstation features a Multichannel pipetting head and flexible configurations to increase walk-away time.


**Figure 2: (A)**The Biomek i5 MC automated workstation, **(B)** the BioPhase CE-SDS Protein Analysis kit and **(C)** the BioPhase 8800 system.

The Biomek i5 MC workstation features include:

- 96 head (1 µL - 1200 µL pipetting capability)
- 360˚ rotating gripper with offset fingers
- Guided Labware Setup (GLS)
- DeckOptix Final Check to ensure accurate system setup
- Reagent volume calculations
- 96 channel tips washing for sample processing and bead-based cleanups
- High deck capacity with 25 positions

The BioPhase 8800 system features include:

- 8-capillary cartridge for simultaneous CE analysis
- Equipped with UV and laser-induced fluorescence (LIF) detection modes; both detectors can be used in the same sequence
- Easy and intuitive front panel control software
- User-friendly method and sequence editor and data analysis software

## Automated Method

Automation provides increased efficiency and reduction in human errors, with minimal hands-on time **(Table 1)**. Please note, these timings are for a 300 µL MC head. With a switch to 1200 µL MC head, the preparation time can be significantly reduced.

**Table 1:** Estimated run time for automating the BioPhase CE-SDS Protein Analysis kit on the Biomek i5 MC Workstation (using a 300µL MC head).

| Metric | Value |
|---|---|
| Sample Throughput | 96 |
| Hands-On Time | 15 minutes |
| Sample Outlet Plate Prep | 57 minutes |
| Reagent Outlet Plate Prep | 39 minutes |
| Reagent Inlet Plate Prep | 18 minutes |
| Sample Prep and Sample Inlet Plate Prep | 1 hour |
| Total Run Time | 3 hours |
| Number of User Interactions | 2 |

The method can be run using the Method Option Selector **(Figure 3)**, Guided Labware Setup to aid with deck setup and reagent calculations **(Figure 4)** and DeckOptix Final Check software to minimize costly setup errors. Automated methods provide flexibility to users in scheduling their workflow and allowing method customizations for sample processing and throughput.

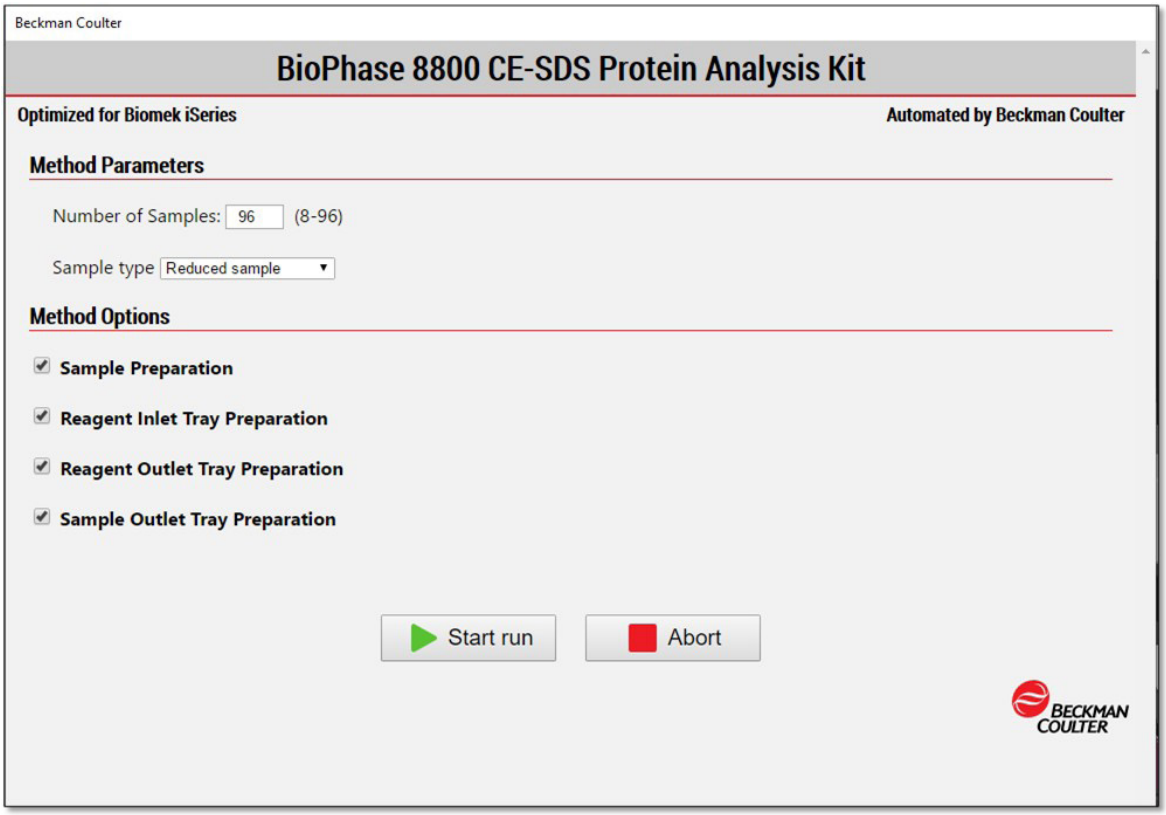


**Figure 3:** The BioPhase CE-SDS Protein Analysis kit automated Method Option Selector (MOS) enables users to select sample number, sample type and method options for sample prep, inlet and outlet trays.

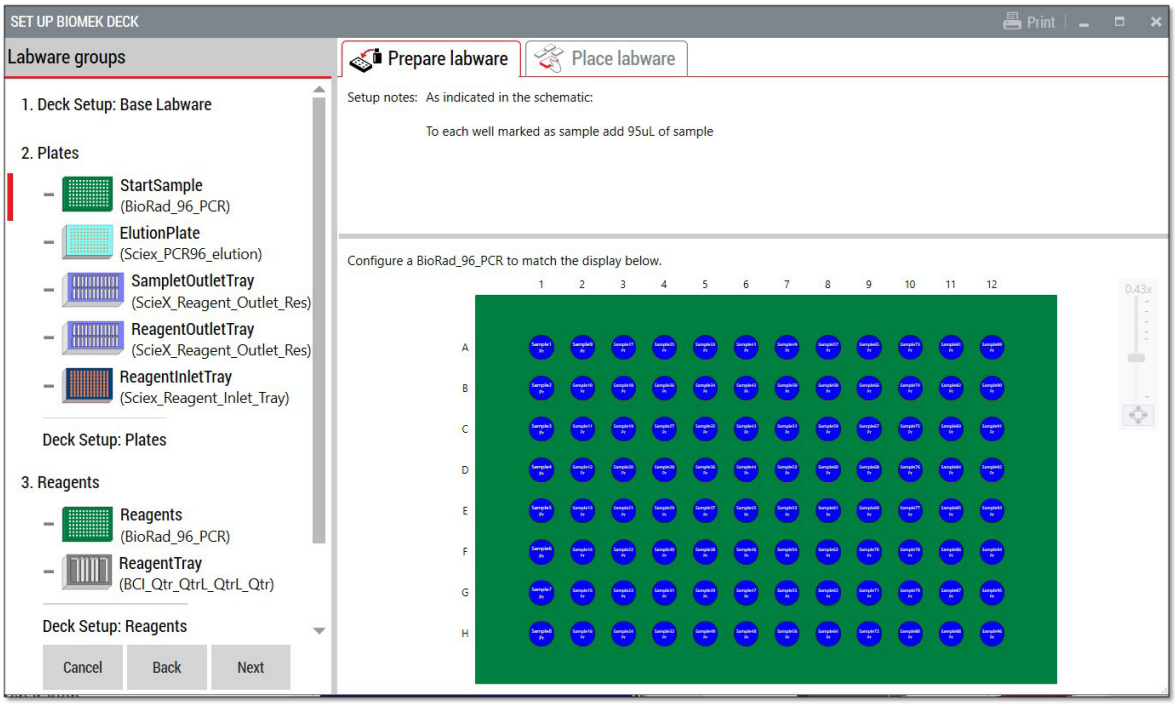


**Figure 4:** Guided Labware Setup (GLS) provides reagent volumes calculated by sample number, preparation notes and pictorial guide for user deck setup.

## Experimental Design

Materials: The BioPhase CE-SDS Protein Analysis kit (PN C30085), including the SDS-MW separation gel buffer, 10 KDa internal standard, basic wash, acidic wash, sample buffer, IgG control standard (PN 391734) and SDS-MW size standard (PN A22196) were from SCIEX (Framingham, MA). The β-mercaptoethanol (β-ME) (PN M3148-25ML) was from Sigma Aldrich (St. Louis, MO). USP monoclonal IgG system suitability (PN 1445550) from USP (Rockville, MD) was used as reference standard for testing.

CE Instrumentation: BioPhase 8800 system equipped with UV absorbance detection at 220 nm (PN 5083590F), the BioPhase BFS capillary cartridge - 8 x 30 cm (PN 5080121) and the starter kit of sample and reagent plates (PN 5080311) were from SCIEX (Framingham, MA).

### Experimental Design

To demonstrate method capabilities for a 96-sample preparation on a Biomek i5 MC workstation, we started with 95 µL of USP IgG SS standard in a 96-well plate. To the sample 2 µL of internal standard and 5 µL of reducing agent (β-ME) were added individually. The plate was then sealed with a film and placed back on deck. It was then heated to 70°C for 10 minutes. 90 µL of these samples were then transferred to the sample inlet tray. The sample inlet tray was centrifuged at 177x g for 20 minutes and then placed into the BioPhase 8800 system for electrophoretic separation. For the reagent inlet plate, all reagents were pipetted column-wise as provided in the user guide[1]. For reagent outlet, a single tip is used for each reagent. For sample outlet tray, a tip is used for each well that needs to be filled (depending on the sample number). The reagent inlet and outlet trays and sample outlet trays were prepped with the Biomek i5 MC workstation and then placed directly into the BioPhase 8800 system. The capillary conditioning was performed as per the user guide followed by the separation. To determine intra- and inter-capillary reproducibility, we analyzed the corrected peak area (CPA) %, relative migration time (RMT) of the species of the reduced control IgG, the ratio of CPA between the heavy chain (HC) and light chain (LC) and the resolution between the peaks of the non-glycosylated heavy chain (ng-HC) and HC.

## Results

We demonstrate an inter-capillary reproducibility better than 0.24 relative standard deviation (RSD) for RMT and 0.38 for CPA % for the HC peak of reduced IgG control standard. For intra-capillary reproducibility, our results showed an RSD for RMT between 0.0% and 0.28% and between 0.21% and 0.47% for CPA%.

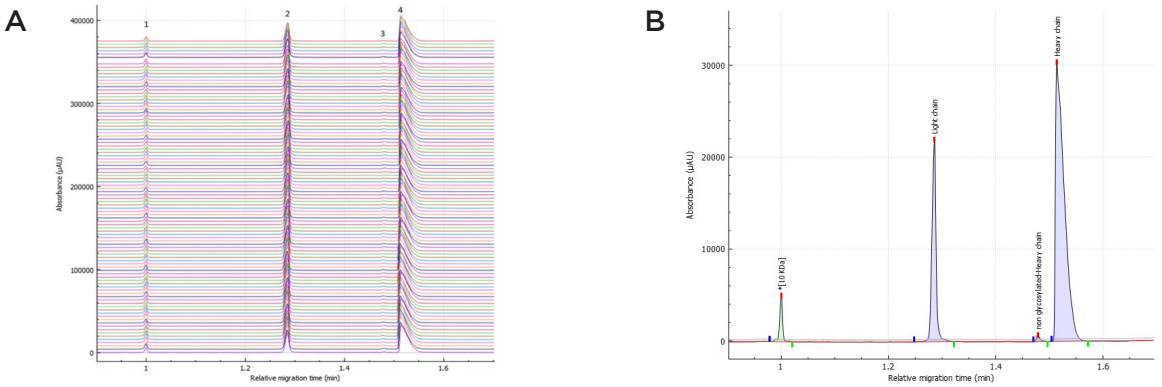


**Figure 5: (A)** CE-SDS electrophoresis of 96 reduced monoclonal antibodies. Peak 1 is the 10 KDa marker, Peak 2 is the light chain (LC), Peak 3 is the non-glycosylated heavy chain (NGHC) and Peak 4 is the heavy chain. The different colors (A-H) denote the 8-capillary numbers with first run starting at the top. **(B)** Electrogram from a single run showing the 4 peaks.

Intra and inter-capillary reproducibility using IgG control standard:

Figure 5A shows the overlayed electropherograms of the IgG control standard for all 96 samples. Traces of absorbance are shown as a function of relative migration time in separate lines of each panel for capillaries A-H and wells A1 to H12.

Migration Time (MT) and RMT: Figure 5A demonstrates a good overlay of the 10 KDa internal standard that was used to calculate the relative times of LC, ng-HC and HC. Table 2 summarizes the intra- and inter-capillary average values for MT. Overall, the average values (N=96) for MT were consistent for inter- and intra-capillaries, indicating the assay's specificity and reproducibility, and the robustness of the cartridge cooling features of the BioPhase 8800 system. Table 3 highlights a %RSD better than 0.8% for the MT for the 3 major species of reduced IgG control standard. The RMT for the 3 species and the 10 KDa marker are plotted for all 96 samples in Figure 6A.

Corrected peak area %: The CPA% was determined automatically by the BioPhase software. Briefly, the CPA% was calculated by dividing the peak area by the velocity of the corresponding peak. The average values for the CPA% of the major species found in the reduced IgG control standards (LC, ng-HC and HC) are displayed in Table 2. Consistent values for CPA% were observed for the reduced form of the IgG control standards across all 96 samples.

| Intra-capillary %RSD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Migration time (mins) | | | | Corrected peak area % | | | Ratio |
| | 10 KDa | LC | ng-HC | HC | LC | ng-HC | HC | HC/LC |
| Capillary A | 0.69 | 0.67 | 0.66 | 0.65 | 0.21 | 2.15 | 0.07 | 0.43 |
| Capillary B | 0.56 | 0.52 | 0.52 | 0.50 | 0.23 | 1.77 | 0.08 | 0.34 |
| Capillary C | 0.55 | 0.51 | 0.50 | 0.48 | 0.36 | 2.21 | 0.13 | 0.38 |
| Capillary D | 0.64 | 0.61 | 0.60 | 0.59 | 0.21 | 2.71 | 0.09 | 0.38 |
| Capillary E | 0.74 | 0.73 | 0.73 | 0.70 | 0.34 | 2.23 | 0.14 | 0.34 |
| Capillary F | 0.76 | 0.74 | 0.72 | 0.71 | 0.22 | 2.00 | 0.08 | 0.27 |
| Capillary G | 0.96 | 0.93 | 0.93 | 0.92 | 0.14 | 1.77 | 0.05 | 0.24 |
| Capillary H | 1.11 | 1.10 | 1.11 | 1.09 | 1.12 | 1.82 | 1.04 | 0.44 |
| Inter-capillary %RSD | 0.83 | 0.81 | 0.82 | 0.80 | 0.47 | 2.24 | 0.38 | 0.37 |

**Table 2:** Intra- and inter-capillary average values for migration time, corrected peak area % and HC/LC ratio for 10 KDa Marker and IgG control standard. N=96

| Intra-capillary average | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Migration time (mins) | | | | Corrected peak area % | | | Ratio |
| | 10 KDa | LC | ng-HC | HC | LC | ng-HC | HC | HC/LC |
| Capillary A | 13.18 | 16.95 | 19.5 | 19.96 | 28.19 | 0.75 | 71.06 | 2.52 |
| Capillary B | 13.13 | 16.89 | 19.43 | 19.89 | 28.14 | 0.75 | 71.10 | 2.53 |
| Capillary C | 13.13 | 16.88 | 19.42 | 19.88 | 28.17 | 0.74 | 71.08 | 2.52 |
| Capillary D | 13.12 | 16.87 | 19.4 | 19.85 | 28.15 | 0.74 | 71.09 | 2.53 |
| Capillary E | 13.17 | 16.94 | 19.48 | 19.95 | 28.2 | 0.74 | 71.05 | 2.52 |
| Capillary F | 13.16 | 16.90 | 19.46 | 19.92 | 28.22 | 0.75 | 71.02 | 2.52 |
| Capillary G | 13.19 | 16.96 | 19.51 | 19.78 | 28.24 | 0.75 | 71.00 | 2.51 |
| Capillary H | 13.28 | 17.08 | 19.65 | 20.12 | 28.09 | 0.74 | 70.86 | 2.52 |
| Inter-capillary Average | 13.17 | 16.93 | 19.48 | 19.92 | 28.18 | 0.75 | 71.03 | 2.52 |

**Table 3:** Intra- and inter-capillary %RSD for migration time, corrected peak area % and HC/LC ratio for 10 KDa marker and IgG control standard. N=96

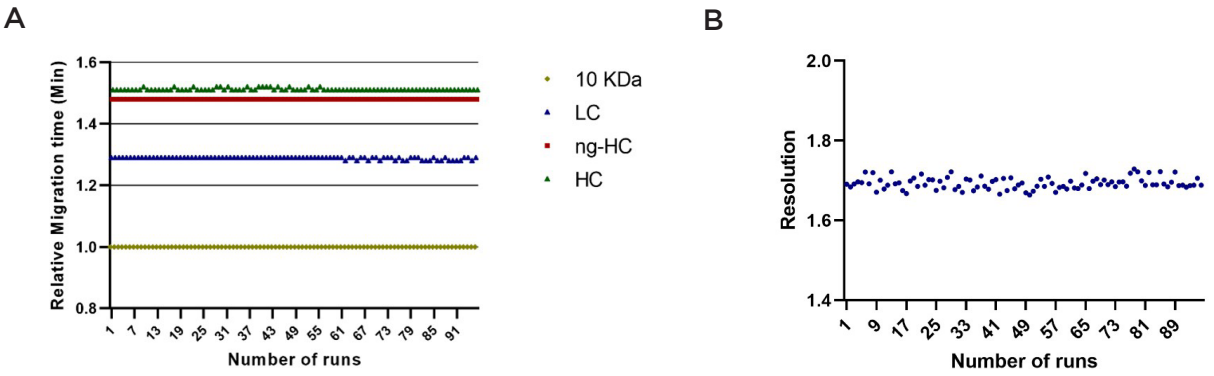


**Figure 6: (A)** Relative Migration Time (RMT) for the 10 KDa marker, LC, ng-HC and HC for all 96 samples. **(B)** Resolution between the ng-HC and HC peak for 96 samples.

HC/LC ratio: The average intra-capillary values are shown in Table 2 and indicate the consistency of the data. A %RSD of the HC/LC ratio between 0.24 and 0.44% was observed for intra-capillary and 0.37% for inter-capillary, indicating good reproducibility (Table 2).

Resolution ng-HC and HC: Resolution between the ng-HC and HC is a critical parameter because because the presence of ng-HC is considered a product-related impurity, as it alters the stability and effector functions of the final therapeutic product. Therefore, it is essential to separate and quantify this variant consistently. The mobility of these species is very similar, and therefore it is necessary to monitor resolution and specificity during the CE-SDS separation under reduced conditions. Figure 6B shows the resolution obtained across 96 individual sample preps. The overall reproducibility achieved for the resolution between the ng-HC and HC resolution is better than 0.87 %RSD.

## Conclusion

- Automated sample and reagent inlet and outlet plate preparation on the Biomek i5 MC workstation eliminates labor-intensive bench work and provides a higher throughput for SDS analysis with highly consistent results when combined with the BioPhase 8800 system.
- Robust automated sample preparation yielded excellent intra-capillary reproducibility data for migration time, relative migration time and corrected peak area percentage compared to manual prep[2].
- Inter-capillary reproducibility values for % RSD for CPA% were less than 0.47, 2.24 and 0.38 for LC, ng-HC and HC respectively.
- Inter-capillary reproducibility values for % RSD for RMT were less than 0.27, 0 and 0.21 for LC, ng-HC and HC respectively.

## References

1. CE-SDS Protein Analysis Kit For the BioPhase 8800 System Application Guide, SCIEX, RUO-IDV-05-8662.

2. SCIEX (2022). Intermediate precision study of capillary electrophoresis sodium dodecyl sulfate (CE-SDS) assay on the BioPhase 8800 system.

The SCIEX BioPhase 8800 system and CE-SDS Protein Analysis kit are for Research Use Only.

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