Maurice® CE-SDS Application Guide

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Introduction

The Maurice CE-SDS Application Kit and Maurice CE-SDS PLUS Application Kit include all you need for your size application development! The kits include the CE-SDS or CE-SDS PLUS cartridges, all sample preparation reagents and consumables that'll let you tackle any monoclonal antibody. This Application Guide will help you every step of the way.

CE-SDS Size Application Kit contents (P/N PS-MAK02-S)

INCLUDES	QTY/KIT
Maurice CE-SDS Cartridges	2
Maurice CE-SDS Cartridge Cleaning Vials	2
Maurice clear screw caps for sample and reagent vials	100
Maurice glass reagent vials, 2 mL	100
Maurice CE-SDS orange pressure caps	12
Maurice 96-well plates	10
Maurice CE-SDS Separation Matrix	15 mL
Maurice CE-SDS Running Buffer – Top	10 vials
Maurice CE-SDS Running Buffer – Bottom	15 mL
Maurice CE-SDS 1X Sample Buffer	25 mL
Maurice CE-SDS Wash Solution	2 x 20 mL
Maurice CE-SDS Conditioning Solution 1	20 mL
Maurice CE-SDS Conditioning Solution 2	20 mL
Maurice CE-SDS 25X Internal Standard	2 vials

CE-SDS PLUS Size Application Kit contents (P/N PS-MAK03-S)

INCLUDES	QTY/KIT
Maurice CE-SDS PLUS Cartridges	2
Maurice CE-SDS PLUS Cartridge Cleaning Vials	2
Maurice clear screw caps for sample and reagent vials	100
Maurice glass reagent vials, 2 mL	100
Maurice CE-SDS orange pressure caps	12
Maurice 96-well plates	10
Maurice CE-SDS Separation Matrix	15 mL
Maurice CE-SDS Running Buffer – Top	10 vials
Maurice CE-SDS Running Buffer – Bottom	15 mL
Maurice CE-SDS PLUS 1X Sample Buffer	25 mL
Maurice CE-SDS Wash Solution	2 x 20 mL
Maurice CE-SDS Conditioning Solution 1	20 mL
Maurice CE-SDS Conditioning Solution 2	20 mL
Maurice CE-SDS 25X Internal Standard	2 vials

If you add the IgG Standard, which is produced with a known quantity of non-glycosylated heavy chain, you can test resolution and quantitation suitability to make sure your Maurice is ready to go.

Storage conditions

- Store the 25X Internal Standard (P/N 046-144) at 2-8 °C.
- Separation and Running buffer bottles should be tightly closed to prevent evaporation.
- Running Buffer – Top vials must be stored stored at 2-8° C when not in use, and tightly closed in the original container with two humidifying pouches.

Ordering info

These kits can be reordered by:

- **Phone:** 1-888-607-9692, option 1
- **Fax:** 1-408-520-4831
- **Email:** orders@proteinsimple.com

Other things you'll need

- Maurice CE-SDS IgG Standard, PN 046-039
- Maurice CE-SDS Molecular Weight Markers, PN 046-432 (optional)
- Maurice sample vials with integrated inserts, 0.2 mL, PN 046-083 (optional)
- β-mercaptoethanol (>98% = 14.2 M) for reducing conditions
- Iodoacetamide (250 mM) for alkylation at non-reducing conditions
- Deionized (DI) water
- Pipettes and tips
- Microcentrifuge and tubes
- Ice and ice bucket
- Vortexer
- Water bath or thermocycler
- Centrifuge with plate adapter or vial adapter (12 mm, 2 mL vials)

Guidelines for great results

- Make sure you read the entire Application Guide before getting started.
- Use fresh Conditioning Solutions, Separation Matrix, and Wash Solution for each batch.
- When the Top Running Buffer vial is still in the cartridge insert, the cartridge **MUST** be kept in an upright position at all times.
- The 25X Internal Standard, IgG Standard, and CE-SDS Molecular Weight Markers are lyophilized. Always reseal the foil bag containing the unopened tubes with desiccant to prevent moisture absorption.
- Aliquot the reconstituted 25X Internal Standard solution into appropriately sized vials and store at -80 °C for long-term storage. For short-term storage (<1 week), the solution can be stored at 2–8 °C.
- If you observe any precipitation in the 25X Internal Standard solution, then leave the solution at room temperature and stir gently until the precipitates have dissolved completely.
- Don't reuse reagents, vials or the clear screw caps. Always keep the orange pressure caps paired with their respective reagents.
- Orange pressure caps should be used for the CE-SDS application only.
- If you want to reuse the orange pressure caps, wash them thoroughly with DI water, soak them overnight in DI water, rinse caps thoroughly with DI water again and air dry them before reusing.
- Whenever you handle the cartridge or remove it from its packaging, make sure the cartridge inlet doesn't come in contact with any surfaces. A damaged inlet may compromise the cartridge and cause a failed injection.

- Always perform the cartridge cleanup before storing, and always store the cartridge in its original packaging at room temperature.
- If you see any Separation Matrix sticking to the cartridge inlet, soak the inlet for 5 min in DI water. Then wipe the inlet using a lint-free laboratory wipe that's been moistened with DI water.
- Each cartridge is guaranteed for up to 100 injections and maxium of 25 batches. A CE-SDS Cartridge has an absolute maximum of 200 injections and a CE-SDS PLUS Cartridge 500 injections. Its RFID will keep track of how many injections have been performed and how many are left for you.

Application overview

A successfully defined and optimized CE-SDS method gives you:

- A highly reproducible peak profile
- Reproducible relative migration times for each peak
- NGHC/HC baseline peak resolution (≥1.5) for the reduced IgG sample

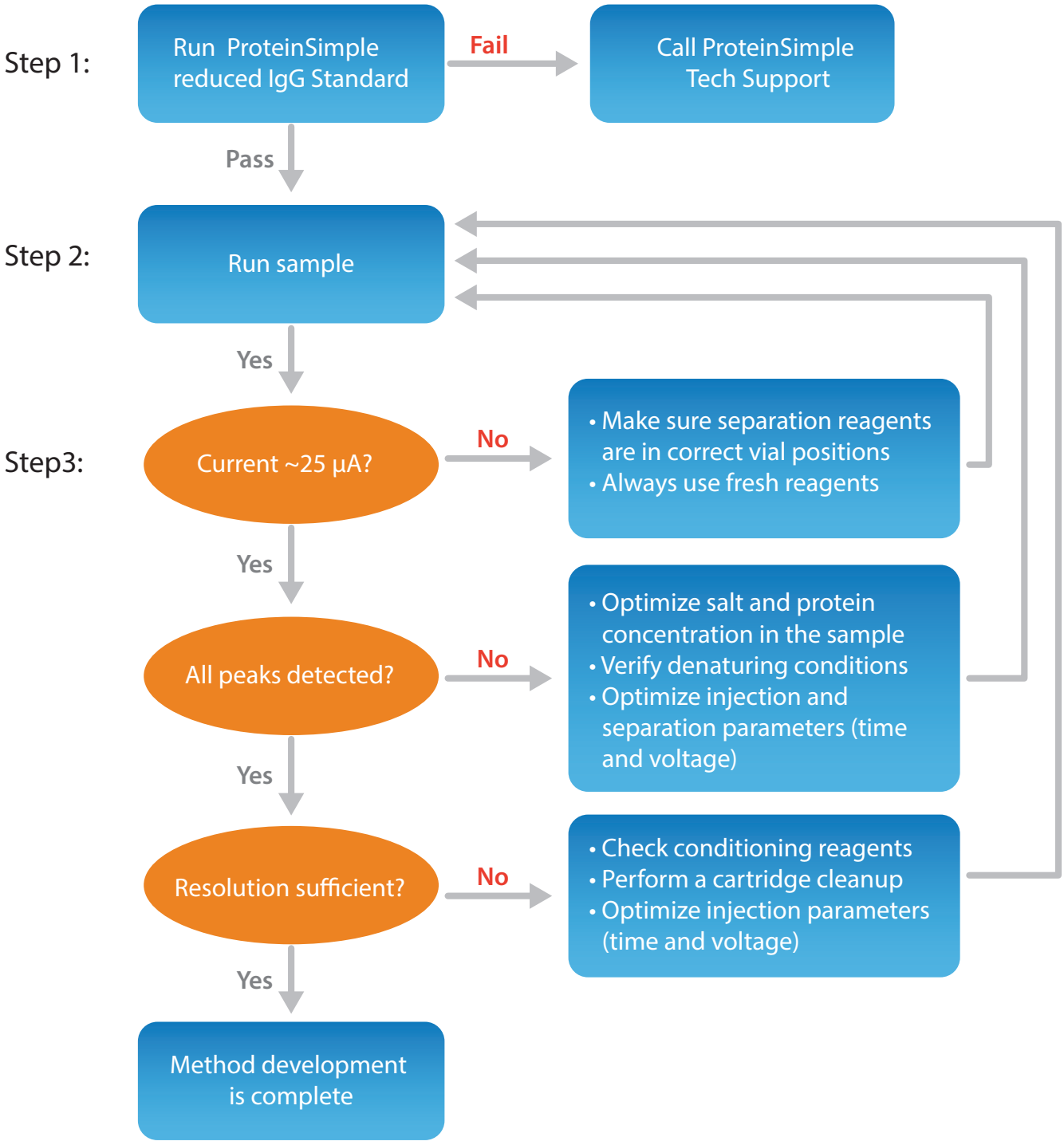

FIGURE 1. Method development strategy.

Step 1: Basic Maurice CE-SDS system performance check

MAURICE SETUP AND START

The IgG Standard allows you to check system performance before you begin running your samples.

Notes:
See Appendices A, B and D for procedures on prepping the 25X Internal Standard and IgG Standard, batch reagents and CE-SDS cartridge prep, respectively.

If you won't use the 25X Internal or IgG Standards after reconstitution, keep them on ice.

To run a system performance check:

1. Prepare your batch reagents and place them in Maurice.
2. Prepare your 25X Internal Standard.
3. Prepare your IgG Standard (reduced and/or non-reduced).
4. Pipette your prepped IgG Standard samples into the 96-well plate.
5. Spin the plate down for 10 min at 1000 xg.
6. Place the 96-well plate metal insert in Maurice and then place your sample plate in the insert.
7. Fetch a fresh vial of Top Running Buffer from 2–8 °C storage and slide the Top Running Buffer vial into the cartridge insert using the procedure in Appendix D, then install the cartridge in Maurice.
8. Launch Compass for iCE.
9. Click the **Batch** screen.
10. In the File menu, click **New Batch**. If your Maurice runs both size and charge, select **Maurice CE-SDS** or **Maurice CE-SDS PLUS** depending on the cartridge type.
11. Add samples by highlighting the sample location(s) in the Layout pane and clicking **Add**.
12. Assign the appropriate default method (Reduced IgG or Non-reduced IgG) to the IgG Standard sample(s) by clicking the **Methods** drop down menu in the Injections pane (see Table 1 for default run conditions).
13. Reinjections are on by default. Click the **Reinject** icon to toggle it off if you don't want Compass for iCE to pause the separation if an abnormal current profile is detected and reinject the same sample automatically.
14. If you want to add sequential replicate injections, highlight the injection in the Injections pane and click **Replicate**.
15. Save your batch.
16. Click **Start**.

	REDUCING CONDITIONS		NON-REDUCING CONDITIONS		CE-SDS MW MARKERS	
STEP	VOLTAGE	TIME	VOLTAGE	TIME	VOLTAGE	TIME
Injection	4600 V	20 sec	4600 V	20 sec	4600 V	20 sec
Separation	5750 V	25 min	5750 V	35 min	5750 V	35 min

TABLE 1. Default run conditions for the IgG Standard and CE-SDS Molecular Weight Markers.

EXPECTED RESULTS

Reduced Samples

A successful result with the reduced IgG Standard should give you four peaks corresponding to the Internal Standard, light chain, non-glycosylated heavy chain and glycosylated heavy chain as shown in Figure 2 (left). When viewing data with the ***Standards*** view selected, you can define the appropriate range of the migration time for the Internal Standard, light chain, and heavy chain using your Maurice system. A general guideline is provided in Table 2 for your reference. When viewing data with the ***Samples*** view selected, the non-glycosylated and glycosylated heavy chains should be baseline resolved with resolution ≥1.5.

PEAK TYPE	MIGRATION TIME (SECONDS)
Internal Standard (reduced and non-reduced IgG)	650 – 800
Light chain (reduced IgG)	800 – 1000
Glycosylated heavy chain (reduced IgG)	1000 – 1300
Intact IgG (non-reduced IgG)	1500 – 1900

TABLE 2. Expected migration times for peaks in reduced and non-reduced IgG Standards.

Non-reduced Samples

In the non-reduced IgG Standard, only three major peaks should be present corresponding to the Internal Standard, non-glycosylated IgG and glycosylated IgG. When viewing data with the ***Standards*** view selected, the migration time for the Internal Standard and intact IgG (non-glycosylated + glycosylated) should be within the ranges listed in Table 2. Up to eight small peaks corresponding to various IgG fragments may also be present depending on the degree of antibody fragmentation. The percentage intact IgG should be >85%.

If you don't see any peaks, or only see a single amorphous peak, please contact ProteinSimple Technical Support at support@proteinsimple.com.

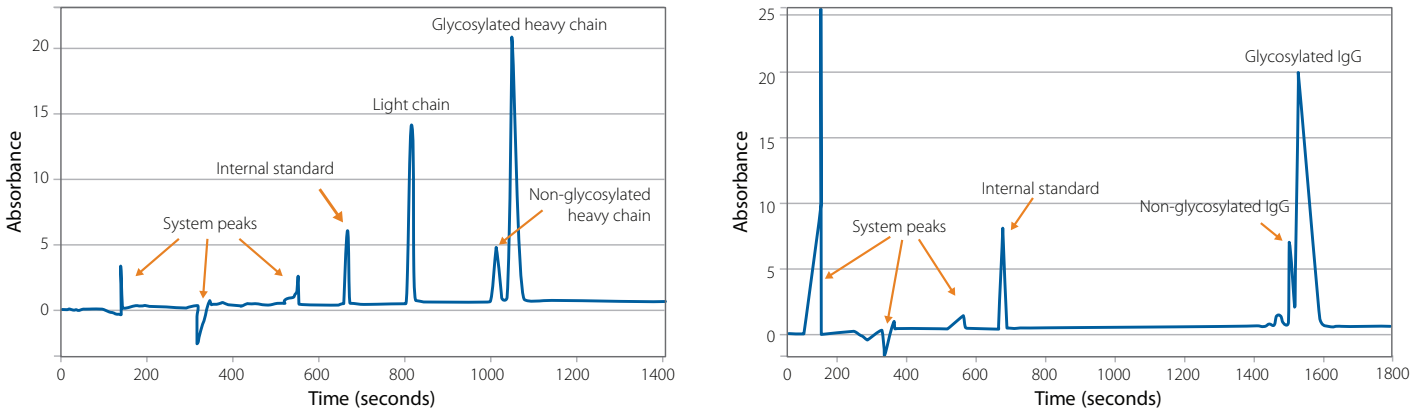

FIGURE 2. Expected results for the IgG Standard under reduced (left) and non-reduced (right) conditions.

Step 2: Set up and run your sample batch

GENERAL CONSIDERATIONS

- For complete details on sample prep see Appendix A.
- Optimal protein concentration depends on sample buffer composition. In general the acceptable concentration is between 0.2 and 2 mg/mL with an optimal salt concentration <50 mM. For example, you can dilute a 5 mg/mL sample with 100 mM NaCl 1:5 into the CE-SDS 1X Sample Buffer, so you'll have 1mg/ mL protein and 20 mM NaCl in the final mix.
- If your sample has low protein concentration, the presence of high salt will decrease sensitivity. In this case, we recommend desalting the sample to obtain the desired sensitivity. See Appendix C for the procedure.
- Sample composition may result in lower electrokinetic injection efficiency and signal decrease. See "Resolution and Signal Intensity Optimization" on page 9 for guidance.
- If molecular weight determination is needed, run the CE-SDS Molecular Weight Markers in your batch.
- You can run as many as 48 injections in one batch. Each sample can be injected once or multiple times, creating replicates. We recommend no more than 12 injections from one sample.

COMMON STEPS

***Note:** If you won't use the 25X Internal Standard, IgG Standard, CE-SDS Molecular Weight Markers or samples immediately, keep them on ice.*

1. Prepare your 25X Internal Standard using the procedure in Appendix A.
2. Dilute samples to 0.25-1 mg/mL in 1X Sample Buffer of your choice (CE-SDS or CE-SDS PLUS). We recommend samples be diluted at least 1:1 with 1X Sample Buffer. See Appendix A for full sample prep details.
3. Add 2 µL of 25X Internal Standard per 50 µL of sample.

FOR REDUCING CONDITIONS

Denature and reduce your samples with β-mercaptoethanol, following the instructions for sample prep in Appendix A. Alternatively, β-mercaptoethanol can be substituted with 10 mM TCEP (tris(2-carboxyethyl) phosphine), using the same denaturing/reducing conditions.

FOR NON-REDUCING CONDITIONS

Alkylate your samples with 250 mM iodoacetamide (IAM) and denature, following the instructions for sample prep in Appendix A.

PREPARATION OF THE CE-SDS MOLECULAR WEIGHT (MW) MARKERS (OPTIONAL)

Prepare the CE-SDS MW Markers as described in Appendix A.

MAURICE SETUP AND START

1. Prepare your batch reagents and place them in Maurice. See Appendix B for prep details.
2. Prepare your 25X Internal Standard and samples as described in Appendix A.
3. Optional: Prepare your IgG Standard and/or CE-SDS MW Markers as described in Appendix A.
4. Prepare your sample plate or vials:

   If you're using a 96-well plate:

   a. Transfer 50 µL of each of your samples and IgG Standard to their designated wells in a 96-well plate.

   b. Cover the plate with a lid and spin for 10 min at 1000 xg using a centrifuge plate adapter.

   c. Pop any remaining bubbles in the samples with a clean pipette tip.

   If you're using vials:

   a. Transfer 50 µL of each of your samples and IgG Standard to their designated sample vials with integrated inserts.

b. Close the vials with a clear screw cap.

c. Spin for 10 min at 1000 xg using a centrifuge vial adapter (12 mm, 2 mL vials).

d. Pop any remaining bubbles in the samples with a clean pipette tip.

5. Insert the Top Running Buffer vial into the cartridge insert using the procedure in Appendix D, then install the cartridge in Maurice.

***Note:** When the Top Running Buffer vial is still in the cartridge insert, the cartridge MUST be kept in an upright position at all times.*

6. Launch Compass for iCE.

7. Click the **Batch** screen.

8. In the File menu, click **New Batch**. If your Maurice runs both size and charge, select **Maurice CE-SDS** or **Maurice CE-SDS PLUS** depending on the cartridge type.

9. Add samples by highlighting the sample location(s) in the Layout pane and clicking **Add**.

10. The default methods should be suitable for most IgGs and many other proteins in the 10-270 kDa range, but you can adjust methods to your specific needs. If needed, you can also create your own method(s) to use in the batch. Method parameters such as injection time and voltage, and separation time and voltage can be changed in the Methods pane. Follow these guidelines:

    a. Default injection and separation conditions in the Reduced IgG method are suitable for detection of reduced IgG peaks. To detect peaks in non-reduced IgG samples, the Non-reduced IgG method can be used. Increase the separation time for detection of high molecular weight species.

    b. To detect all CE-SDS Molecular Weight Markers peaks, use the MW Markers method.

    c. For other method parameter changes, see the Method Optimization section.

11. Reinjections are on by default. Click the **Reinject** icon to toggle it off if you don't want Compass for iCE to pause the separation if an abnormal current profile is detected and reinject the same sample automatically.

12. If you want to add sequential replicate injections, highlight the injection in the Injections pane and click **Replicate**.

13. To analyze the same sample with different methods, you can add multiple injections of the same sample using the **Add** button and then assign different methods to each injection.

14. Save your batch.

15. Click **Start**.

Step 3: Evaluate your results

Evaluate your injection profiles:

1. For replicate injections of the same sample, compare the peak profile across injections. Your peak profiles should be reproducible as shown in Figure 3.

2. The Internal Standard migration time in all samples should be <850 s.

3. For reduced IgG, you should typically see baseline resolution between glycosylated and non-glycosylated heavy chain peaks.

4. For non-reduced IgG, you should be able to detect a non-glycosylated IgG peak as low as 1% along with the main intact IgG peak.

5. When looking for impurities, peaks representing 0.1% impurities of the main protein should be detectable.

6. If you ran the CE-SDS Molecular Weight Markers, seven well-defined peaks should be detected as shown in Figure 4.

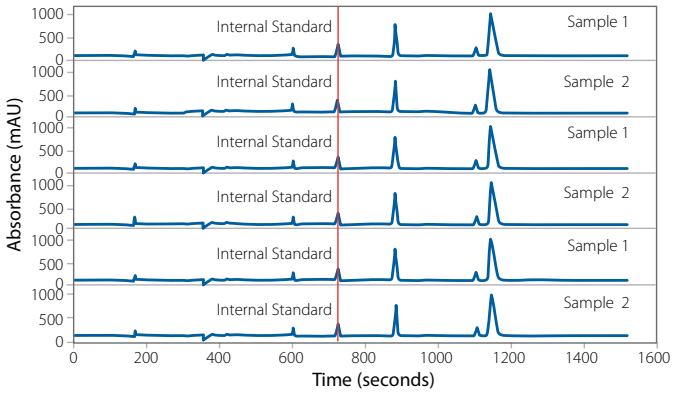

FIGURE 3. Reproducible peak profile in repeated injections from two duplicate reduced IgG samples.

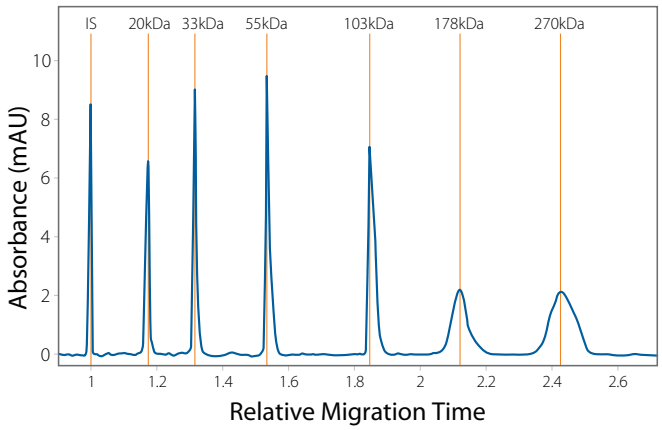

FIGURE 4. CE-SDS Molecular Weight Markers.

Method optimization and troubleshooting

NON-REPRODUCIBLE PEAK PROFILES

- Non-reproducible peak profiles can be a sign of insufficient capillary conditioning. It's critical to use fresh reagents for each batch.
- They can also result from running the cartridge beyond the maximum number of 100 guaranteed injections.

NOISY BASELINE

A noisy or non-flat baseline can be caused by various reasons:

- Insufficient cleaning of the capillary after the batch can produce a noisy or spiky baseline. After a batch is completed, it's necessary to perform the Cartridge Cleanup procedure before you store your cartridge. If the batch was unexpectedly stopped by a user or because of an instrument error, an additional capillary cleaning is required. Perform the CE-SDS Cartridge Purge procedure before you store the cartridge (see the Control chapter in the *Maurice User Guide*).
- Sample precipitation can also cause baseline spikes. Spinning the sample for at least 10 min at 1000 xg minimizes this effect.
- Compass for iCE (V2.1 and later) can apply baseline smoothing to CE-SDS data to decrease noise and improve signal-to-noise ratios. Smoothing is on by default but can be turned off in the Default Analysis Settings before you start a batch or during analysis after a batch is completed.

RESOLUTION AND SIGNAL INTENSITY OPTIMIZATION

Adjusting peak resolution or modifying signal intensity can be achieved by changing injection conditions.

- Decreasing the injection time but keeping the same injection voltage and separation conditions will result in higher resolution, but lower signal.
- Increasing the injection time while keeping the default injection voltage will boost signal intensity, but will negatively affect resolution. However, these conditions may be favorable when you need to detect small amounts of impurities in a sample (0.1% of the main protein and lower).
- Injection times should be optimized by testing increased time in 5-second increments, up to 40 s.
- We don't recommend increasing the injection or separation voltage above the default conditions as this can cause Joule heating and bubble formation.

RESOLUTION TROUBLESHOOTING

A successfully defined and optimized CE-SDS method should give you baseline resolution of the peaks for non-glycosylated and glycosylated heavy chain of the reduced IgG. In a non-reduced IgG sample, you should be able to detect as little as 1% of non-glycosylated IgG.

- Low resolution between peaks in reduced and non-reduced IgG samples may require troubleshooting. Insufficient resolution is often seen in conjunction with long migration time. The most probable cause is insufficient conditioning. It's critical to use new vials and fresh reagents for each batch.
- Low resolution can also be caused by partial clogging of the capillary due to insufficient cleaning. Always perform the Cartridge Cleanup step in Compass for iCE before you store your cartridge. If you see any Separation Matrix on the end of the capillary, gently remove it with lint-free laboratory wipes and soak the cartridge inlet in DI water for 5 min. Then wipe the inlet using a lint-free wipe that's been moistened with DI water. Keep the cartridge in its plastic storage container between uses.

- If a batch was unexpectedly stopped by a user or because of an instrument error, an additional capillary cleaning is required. Perform the CE-SDS Cartridge Purge procedure before you store the cartridge (see the Control chapter in the *Maurice User Guide*).

SEPARATION TIME AND THROUGHPUT OPTIMIZATION (CONSISTENCY)

You can optimize sample separation to reduce batch run time and achieve the highest possible throughput. You can do this in one of two ways:

1. Set up a batch to test your sample at different separation times. Start with 23 min and increase separation time by 2 to 3 minute increments. Choose the shortest separation time that provides acceptable resolution and peak detection.
2. Observe the sample while it's separating. Generally, peaks in the 10-270 kDa molecular weight range pass the detector between 10 and 35 min during separation. You can use the CE-SDS Molecular Weight Markers to estimate the approximate migration time of your protein of interest. If your protein is heavily glycosylated, the migration time may be slower than expected.

Once migration time for the peaks of interest is identified, we suggest adding 2 min to account for slight variabilities in migration time between injections and cartridges. Due to this variability, Relative Migration Time (RMT), and not the absolute migration time should be used to characterize assay variability.

Note: *If you need any assistance optimizing your injection reproducibility, peak resolution or signal intensity, please contact your ProteinSimple Field Application Specialist or our Technical Support team at support@proteinsimple.com.*

Appendix A: Sample and standard preparation

PREPARING THE INTERNAL STANDARD

1. Open the vial of lyophilized 25X Internal Standard by lifting the center tab and gently pulling it back to break the metal seal. Then remove the rubber stopper.
2. Reconstitute by adding 240 µL of 1X Sample Buffer from your Application Kit. Pipette up and down a few times to mix thoroughly. This results in a 25X Internal Standard solution.

Notes:
Don't vortex the lyophilized Internal Standard during preparation.

If you won't be using the Internal Standard immediately, keep it on ice. If you observe any precipitation, leave the solution at room temperature and stir gently until the precipitates have dissolved completely.

PREPARING THE ALKYLATION REAGENT (NON-REDUCED IgG STANDARD AND SAMPLES ONLY)

1. Prepare the alkylation reagent by weighing out 46 mg of iodoacetamide (IAM) directly into a 1.5 mL microcentrifuge tube.
2. Add 1 mL of DI water to the tube and mix thoroughly.

Note: *Prepare a fresh 250 mM solution of iodoacetamide in DI water before use.*

PREPARING THE IgG STANDARD

1. Using scissors, carefully cut the top of the foil package, leaving the sealing strip intact.
2. Take out the strip of tubes and carefully cut one pink tube of lyophilized IgG Standard from the strip. Put the unopened tubes back in the package, seal tightly and store at 2-8 °C.
3. Pierce the foil on the tube with a clean pipette tip.
4. Reconstitute the IgG Standard with 50 µL of 1X Sample Buffer from your Application Kit. Gently resuspend by pipetting the solution up and down. Transfer the solution to a microcentrifuge tube.
5. Add 2 µL of reconstituted 25X Internal Standard.
6. **For a reduced IgG Standard:** Add 2.5 µL of 14.2 M β-mercaptoethanol and mix thoroughly by vortex.
   For a non-reduced IgG Standard: Add 2.5 µL of 250 mM iodoacetamide and mix thoroughly by vortex.
7. Heat the mixture in a water bath or thermocycler at 70 °C for 10 min.
8. Place the tube on ice for 5 min.
9. Vortex briefly and spin down.

PREPARING THE CE-SDS MW MARKERS (OPTIONAL)

1. Using scissors, carefully cut the top of the foil package leaving the sealing strip intact.
2. Take out the strip of tubes and carefully cut one green tube of lyophilized CE-SDS MW Markers from the strip.
3. Put the unopened tubes back in the package, seal tightly and store at 2-8 °C.
4. Pierce the foil on the tube with a clean pipette tip.
5. Reconstitute the CE-SDS MW Markers with 50 µL of 1X Sample Buffer from your Application Kit.. Gently resuspend by pipetting the solution up and down. Transfer the solution to a microcentrifuge tube.
6. Add 2 µL of reconstituted 25X Internal Standard.
7. Add 2.5 µL of 14.2 M β-mercaptoethanol and mix thoroughly by vortex.
8. Heat the mixture in a water bath or thermocycler at 70 °C for 10 min.
9. Place the tube on ice for 5 min.
10. Vortex briefly and spin down.

PREPARE YOUR SAMPLES

1. In a microcentrifuge tube, dilute your IgG sample with 1X Sample Buffer from your Application Kit to a concentration of 0.25-1 mg/mL in a final volume of 50 µL.

Note: *Dilute with Sample Buffer to the desired final concentration. Sample Buffer must constitute at least 50% of the final concentration.*

2. Add 2 µL of reconstituted 25X Internal Standard.

3. **For reduced IgG samples:** Add 2.5 µL of 14.2 M β-mercaptoethanol and mix thoroughly by vortex. **For non-reduced IgG samples:** Add 2.5 µL of 250 mM iodoacetamide and mix thoroughly by vortex.
4. Centrifuge the tube and heat the mixture in a water bath or a thermocycler at 70 °C for 10 min.
5. Put the tube on ice for 5 min.
6. Vortex briefly and spin down.
7. Transfer 50 µL of the sample to a 96-well plate.
8. Spin plate for 10 min at 1000 x g using a centrifuge plate adapter.
9. Pop bubbles in your sample with a clean pipette tip.

***Note:** If you won't be using your samples immediately, keep them on ice.*

Appendix B: Reagent preparation

Prepare your batch reagents as shown in Table 3 and place the reagent vials in Maurice as shown in Figure 5. Depending on how you prepped your samples, place the 96-well plate insert or the sample vials insert into Maurice.

REAGENT	VOLUME	CAP	POSITION
Conditioning Solution 1	1.5 mL	**Orange** pressure cap	P1
Conditioning Solution 2	1.5 mL	**Orange** pressure cap	P2
Wash Solution	1.0 mL	**Orange** pressure cap	P5
Wash Solution	1.5 mL	**Clear** screw cap	N1
Wash Solution	1.5 mL	**Clear** screw cap	N2
Separation Matrix	1.0 mL	**Orange** pressure cap	P4
Running Buffer – Bottom	1.0 mL	**Clear** screw cap	N4
DI water	1.5 mL	**Orange** pressure cap	P3
Empty vial (air)	N/A	**Orange** pressure cap	P6

TABLE 3. Batch reagent preparation.

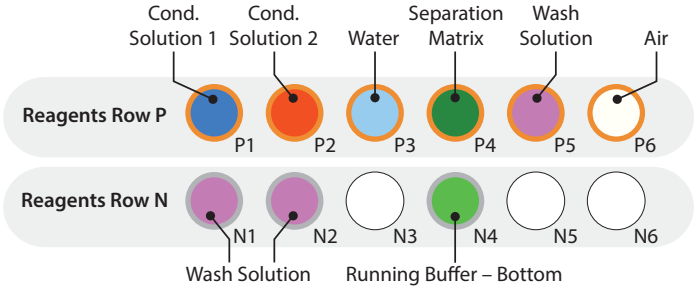

FIGURE 5. Reagent vial placement.

Appendix C: Desalting and concentrating samples

1. Add 500 µL of your sample into an Amicon Ultracel 50K Membrane Centrifugal Filter (Millipore, PN 4311).
2. Centrifuge for 5 minutes at 12,000 xg.
3. Replace the filtered volume with 20 mM Tris buffer pH 7.0 (Life Technologies, PN AM9851).
4. Discard the filtrate from the centrifuge vial.
5. Do two additional cycles of centrifugation and buffer replacement.
6. For simple desalting, replace the filtered volume to 500 µL. If you need to concentrate the sample, store the remaining 100 µL of buffer-exchanged sample at -20 °C or below if you won't use it immediately.

Appendix D: CE-SDS Cartridge preparation

1. Take the cartridge out of its packaging. Save the packaging, you'll need it later.
2. Pull the cartridge insert out of the cartridge.
3. Fetch a fresh vial of Top Running Buffer from 2–8 °C storage and slide the Top Running Buffer vial into the cartridge insert so that the metal pin on the side of the vial is facing out. Press the vial up until it is completely inside the cartridge insert (see Figure 6).

Notes:

The Top Running Buffer vial has metal pins on either side, so no specific orientation is necessary.

Make sure to keep the cartridge insert in an upright position after the Top Running Buffer vial is inside it.

4. Slide the cartridge insert back into the cartridge.

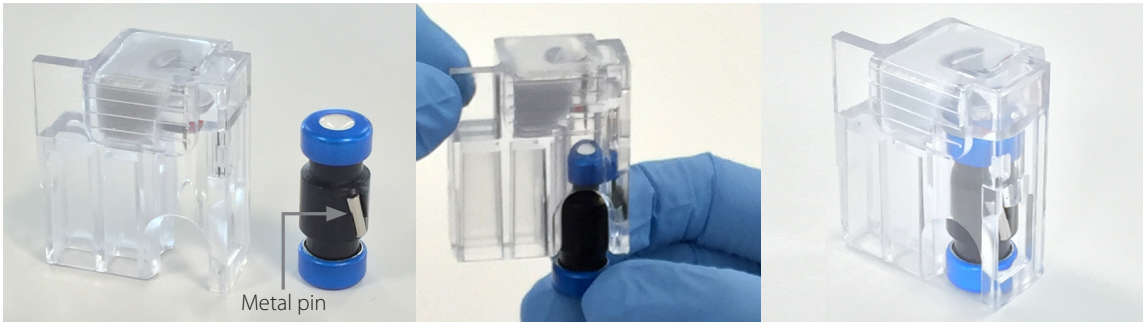

FIGURE 6. Assembling the Top Running Buffer vial in the cartridge insert. Keep the cartridge insert in an upright position after the vial is inside it.

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046-297, Rev F