Selecting an Electrophoresis Time for Multiplexed Single-Cell Western Assays

Introduction

Milo can multiplex, allowing you to detect multiple proteins in each single-cell separation. The SDS-PAGE step separates proteins based on molecular weight and selecting an appropriate time for this step is key to being able to detect all your proteins of interest. On scWest chips, the lanes used for the electrophoretic separation are just 900 microns long, so it is important to select an electrophoresis time for which your largest molecular weight protein makes it fully into the gel, but your smallest molecular weight protein does not run through the end of the lane into the next lane. This tech note provides guidance on selecting an appropriate electrophoresis time when designing multiplexed Single-Cell Western assays.

The How-To

Figure 1 shows the predicted minimum and maximum electrophoresis times for a protein with a specified molecular weight to migrate 200 microns and 750 microns, respectively. The purple lines (solid and dashed) show the minimum recommended electrophoresis time to ensure that your protein(s) of interest have migrated into the gel lane. The blue lines (solid and dashed) show the maximum recommended electrophoresis time to ensure your protein(s) of interest have not run into the next lane. The black dashed line shows average migration halfway through the lane (450 microns).

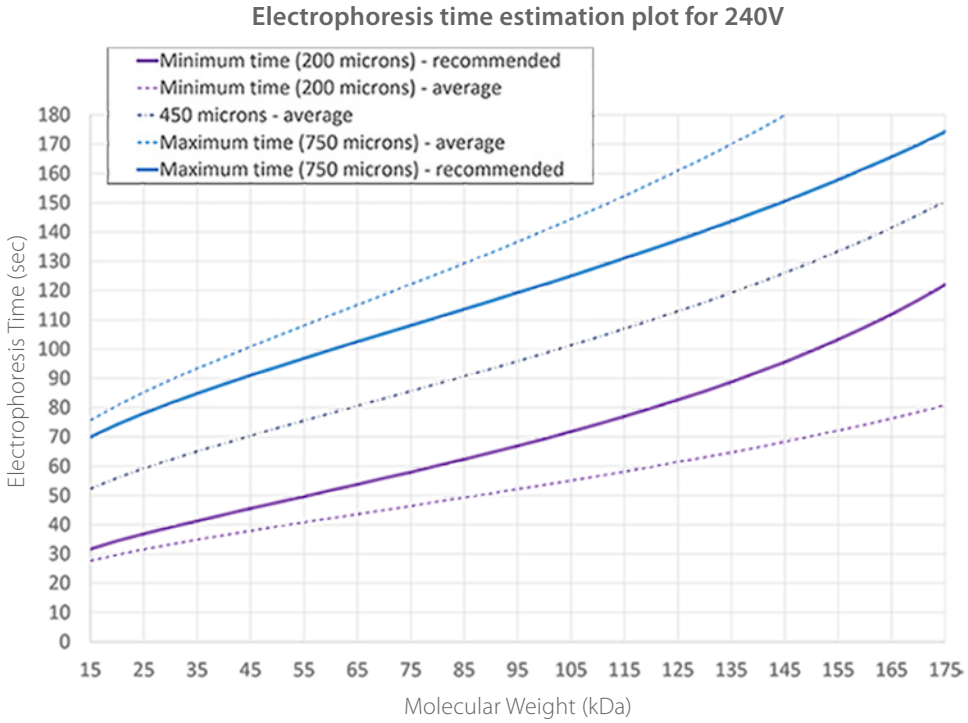


Figure 1. Electrophoresis time estimation plot for electrophoresis run at 240 V.

When designing a multiplexed assay, first identify the molecular weights of the smallest and largest proteins you are looking to detect. Then use **Figure 1** to identify the minimum time that can be used for your largest protein and the maximum time that can be used for your smallest protein of interest. Finally, select an electrophresis time for your experiment that falls between these two times. Where possible, use the solid lines in **Figure 1**; they offer a higher degree of confidence your band will be within this range. If that is not possible for the range of molecular weights you are looking to detect, use the dashed lines which show the average migration distance expected for proteins of that molecular weight and provide a less conservative estimate of protein migration distance (and carry a higher risk that some of your protein bands will be outside the desired range). Importantly, **Figure 1** gives an estimate for an electrophoresis time to start with when establishing an entirely new assay. Variation in migration distance between chips and within a chip and differences between observed and predicted molecular weights of certain proteins mean that you might need to adjust your electrophoresis time after your first run to fully optimize the assay. Electrophoresis times longer than 180 seconds are not recommended and can cause excessive heating.

The solid lines in **Figure 1** show there is not a good overlapping electrophoresis time that allows for the simultaneous detection of proteins greater than 100 kDa with a protein that is 15 kDa without a chance of overrunning the small protein into the next lane. In short, the maximum runtime for a 15-kDa protein corresponds to the minimum runtime for a 100-kDa protein. However, if a protein does run into the next lane, Scout can still accommodate this situation (**Figure 2**). All you have to do is change the start and end location of the detection lane in the Scan Properties window. Nonetheless, protein loss will occur as the protein band migrates through the microwell at the top of the subsequent lane and may interfere with proper detection.

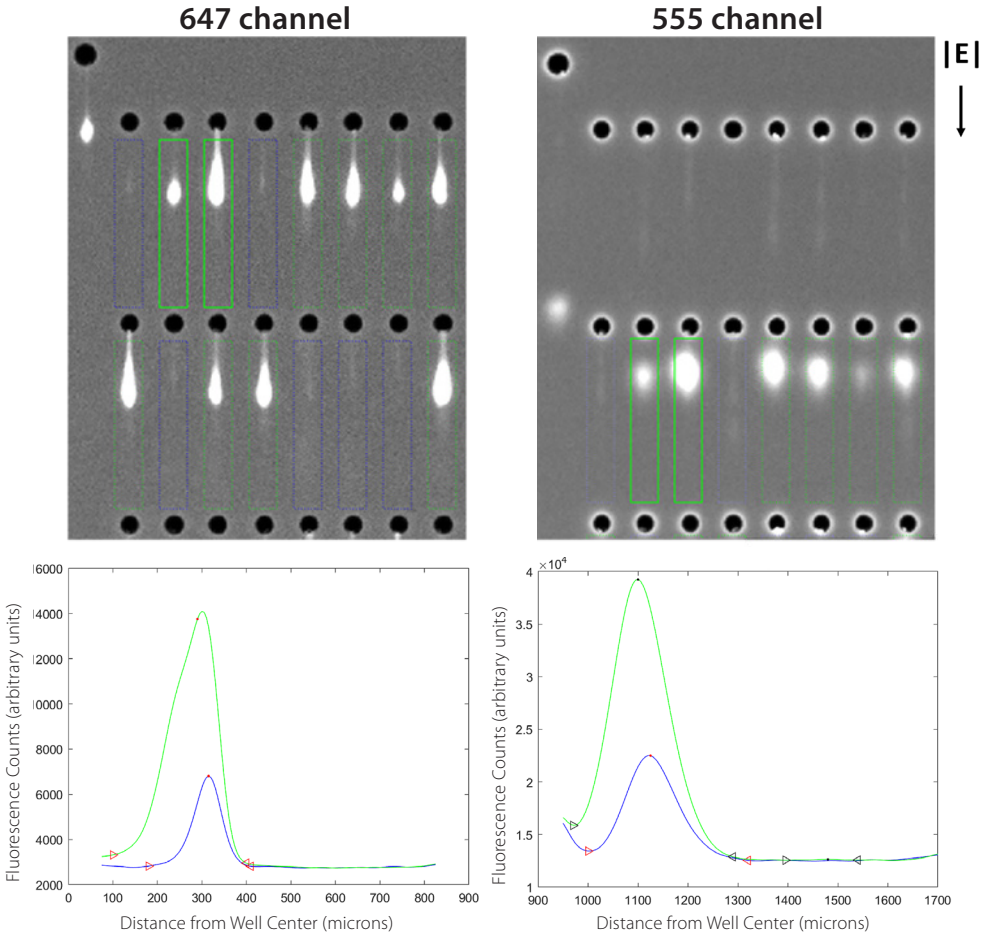


Figure 2. Protein separations imaged in two different spectral channels where one band (555 channel) has overrun into the next lane. By changing the lane start and end locations in the Scan Properties window, Scout can still detect the overrun peak and index it to the originating well. However, selection of the appropriate electrophoresis time can prevent this situation by enabling all proteins of interest to be detected in one lane.

Figure 3 provides an example of how to use **Figure 1** to select an electrophoresis time for a multiplexed experiment aimed at detecting vinculin (124 kDa), β-tubulin (50 kDa) and ERK (44 kDa). The graph shows that the maximum electrophoresis time for ERK is around 90 seconds while the minimum electrophoresis time for vinculin is approximately 80 seconds (using the conservative, solid line estimates). An electrophoresis time of 85 seconds can, therefore, be used as an initial starting point in this experiment to detect all three proteins in the lane.

Figure 4 shows the results of a multiplexed assay detecting Tra98 (146 kDa), DDX4 (76 kDa) and Histone H3 (15 kDa). Using the conservative, solid line estimates in **Figure 4A**, we see that the optimal electrophoresis time is around 70 seconds. **Figure 4B** shows a clear detection of all three protein bands in the lane using a 70-second electrophoresis time.

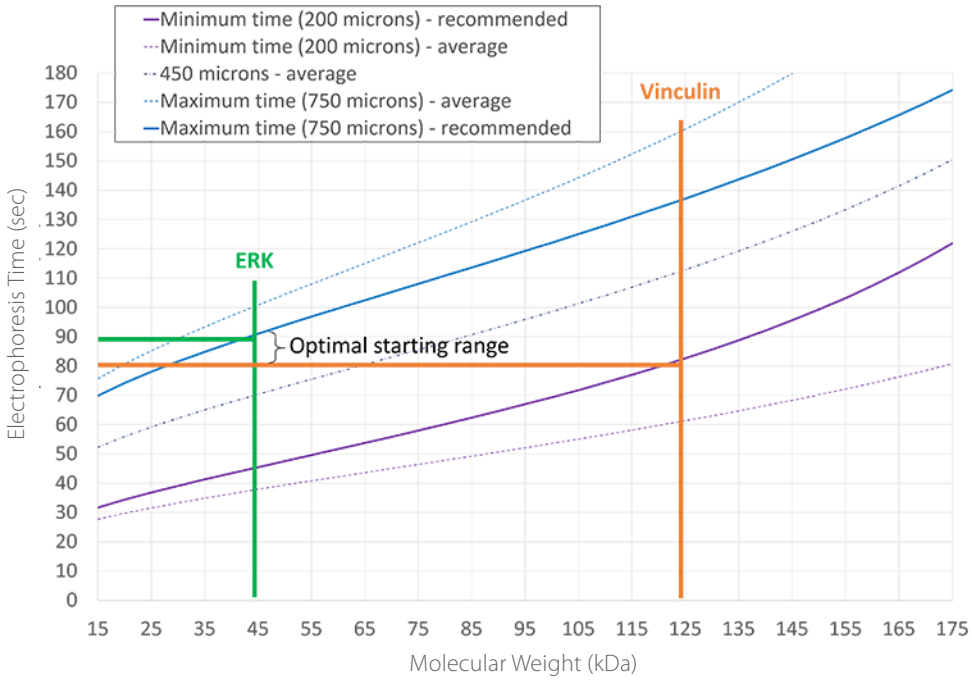


Figure 3. Selection of the optimal electrophoresis time for a multiplexed Single-Cell Western experiment can be made by identifying the minimum electrophoresis time for your largest protein and the maximum electrophoresis time for your smallest protein. Use an electrophoresis time between these minimum and maximum times to detect any proteins with a molecular weight that falls within the range established by the smallest and largest proteins.

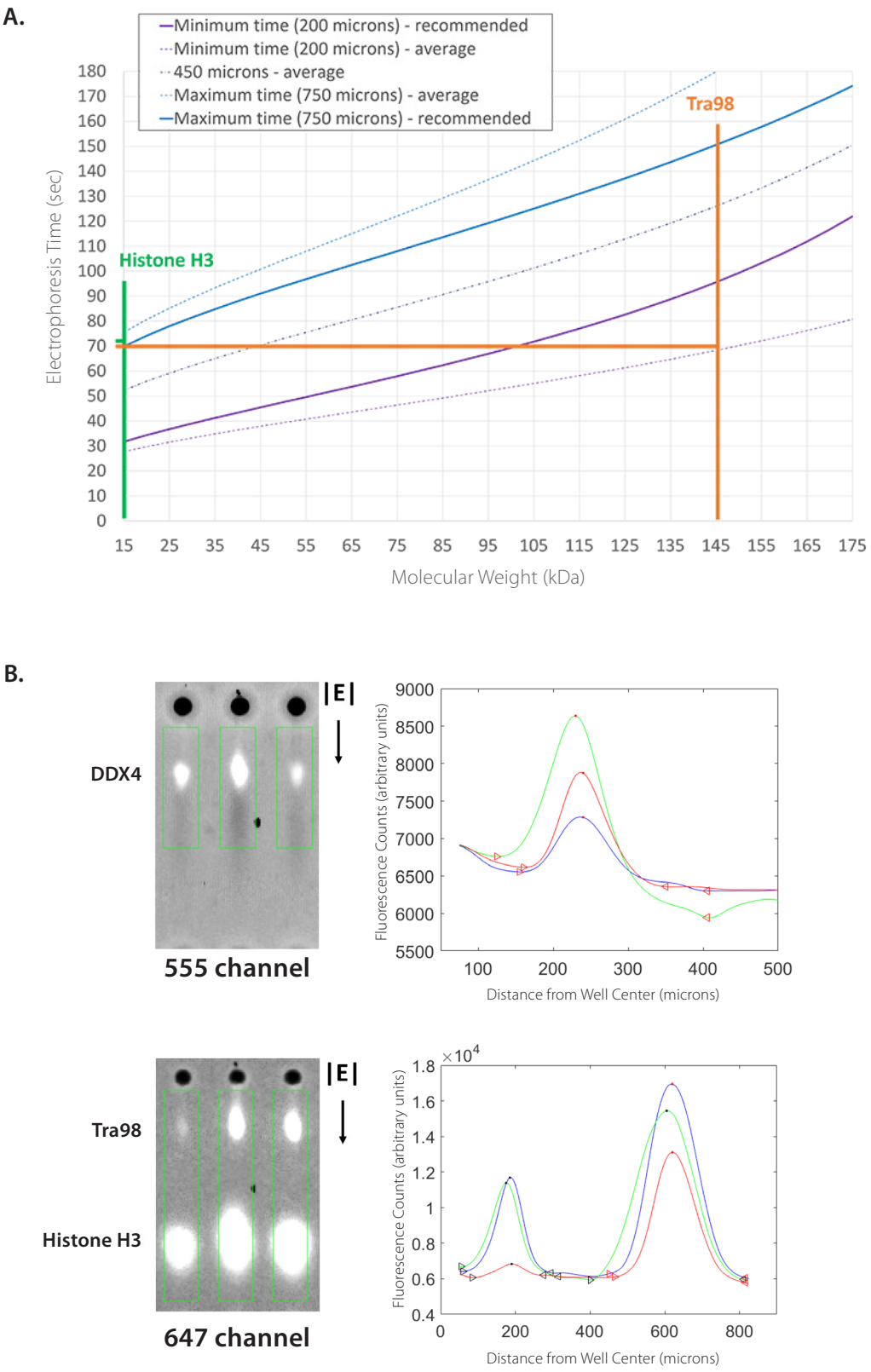
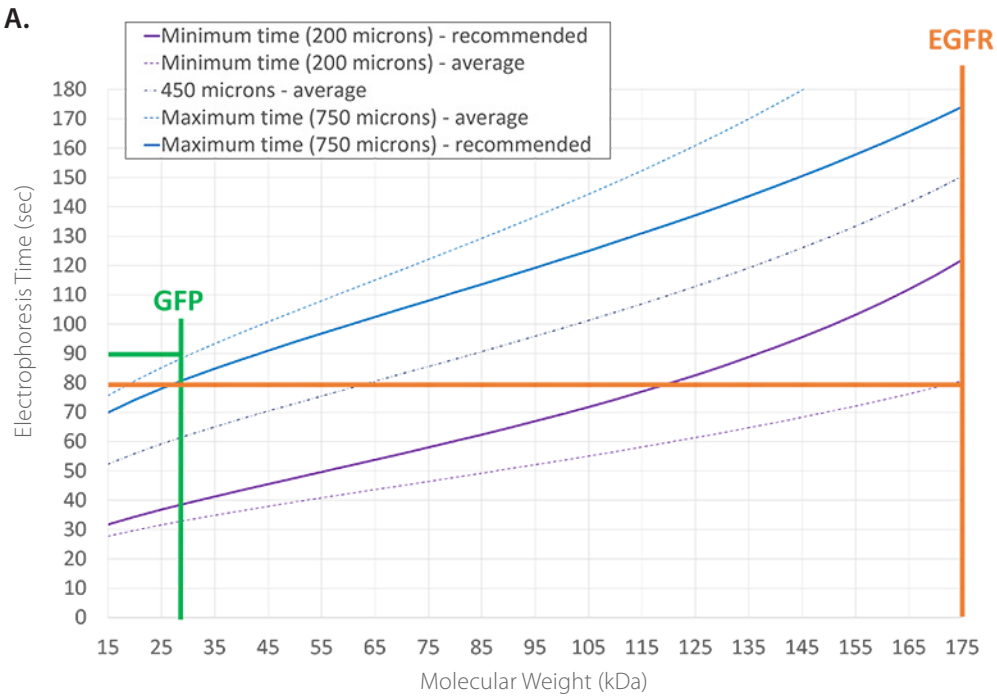


Figure 4. An optimal electrophoresis time of 70 seconds should be selected for a multiplexed assay detecting Histone H3 (15 kDa), DDX4 (76 kDa) and Tra98 (146 kDa) (A). Separations of each protein with a 70-second separation show clear detection of all peaks in the lane (B).

The electrophoresis times given in **Figure 1** should be used as a guide when designing your assay and adjusted as needed based on empirical results. **Figure 5** shows a multiplexed experiment detecting four proteins which vary in molecular weight by almost 150 kDa: EGFR (175 kDa), pAKT (60 kDa), β-tubulin (50 kDa), and GFP (27 kDa). Using the solid lines in **Figure 5A,** we see that there is no overlapping electrophoresis time between 175 kDa and 27 kDa proteins meaning that there is no electrophoresis time that will conservatively position both the 175 kDa and 27 KDa proteins between 200 microns and 750 microns. Without consulting **Figure 5A**, this experiment was run with a 60 second electrophoresis time. **Figure 5B** shows that a 60-second electrophoresis time was sufficient to detect all four protein bands. However, the smallest protein (GFP, 27 kDa) ran only about halfway through the gel (approximately 450 micron separation distance) while the largest protein (EGFR, 175 kDa) is still close to the well (approximately 150 micron separation distance). As expected for a 60 second separation time, β-tubulin (50 kDa) and pAKT (60 kDa) ran at approximately 400 micron and 350 micron migration distances, respectively. While there is no overlapping recommended electrophoresis time for 175 kDa and 27 kDa proteins using the solid lines in **Figure 5A**, if we use the less conservative dashed lines in **Figure 5A**, we can select an improved electrophoresis time of 80-90 seconds to position all four proteins between 200 microns and 750 microns into the gel.



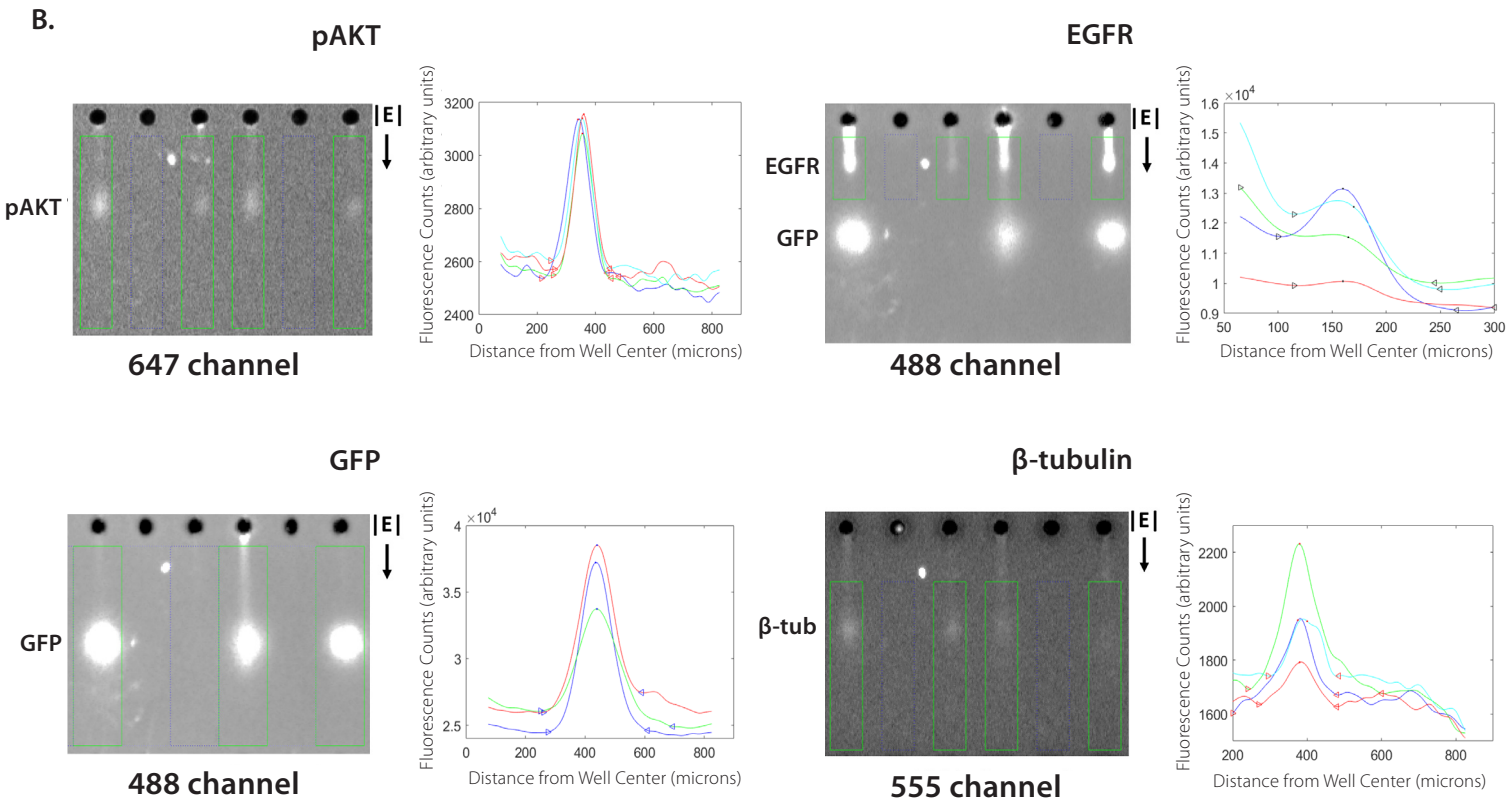


Figure 5. Selection of an electrophoresis time for a four-plexed assay of GFP, pAKT, β-tubulin and EGFR shows an ideal electrophoresis time range of 80 to 90 seconds (A). Separations of each protein with a 60-second separation show clear detection of all peaks in the lane (B). However, the smallest protein (GFP) only runs halfway down the lane, and the largest protein (EGFR) has just entered the gel. A 90-second separation time is recommended for this assay.

Conclusion

Designing multiplexed Single-Cell Western assays starts with selecting an appropriate electrophoresis time that allows your largest molecular weight protein to get into the gel but doesn't allow your smallest molecular weight protein to overrun the lane. Use this guide to help streamline the process of selecting an appropriate electrophoresis time when you're setting up a new assay. Once you've selected an electrophoresis time for your assay, simply enter it into Milo's touchscreen interface and start your experiment!

proteinsimple
a biotechne brand

Toll-free: (888) 607-9692
Tel: (408) 510-5500
info@proteinsimple.com
proteinsimple.com