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SmartLabel Pro - Labeling Protocol

SmartLabel is designed for use with samples preserved using a method such as SHIELD, CLARITY, or SWITCH and that have been delipidated using an SDS-based clearing approach like LifeCanvas's SmartClear 2 Pro.

This is a detailed protocol for using LifeCanvas Technologies' SmartLabel Pro to label samples the size of mouse brain hemispheres. ***Please read the protocol in its entirety before starting an experiment.*** *After studying this detailed protocol, you may find it more convenient while performing experiments to refer to the Labeling Protocol Quick Guide for a more concise list of key steps.*

Reagents needed:

- Labeling-OFF buffer (one bottle, 500 mL total)
- Labeling-ON buffer (one bottle, 1 L total)
- *Sample buffer* (~1.5-4 mL — *depends on sample size and cup used)
- *Sample-WASH buffer* (~7-10 mL — *depends on sample size and cup used)
- Bovine serum albumin ('BSA' -- e.g., Sigma-Aldrich #A7030)
- 20% Triton-X 100 in Labeling-ON buffer solution (prepare a small amount of stock solution, ~10 mL, for use across experiments)
- Labeling reagents, such as primary and secondary antibodies or fluorescent nuclear dyes
- 10 mM SDS + 0.02% sodium azide in DI water solution, for rinsing and storing sample cups
- Optional: thin strips of plastic mesh (e.g., ~5 mm wide by ~40-50 mm long, cut from McMaster cat# 9318T22) to prevent small samples from moving or rotating within the sample cup

Solutions setup:

- **Working volumes of both *Sample* & *Sample-WASH* buffers need to have BSA added to them at a final concentration of 2% weight-to-volume before being used in SmartLabel labeling experiments. All references below to these buffers refer to working solutions to which BSA has already been added.**
- These working solutions should be stored at 4°C and used within one month of being prepared, after which it is recommended to discard them in favor of freshly-prepared solutions.

Recommended set/limit values and [normal operating ranges] for SmartLabel functional parameters:

- Voltage = 90 V [Voltage Control mode attempts to keep voltage fixed at 90 V]
- Current = 1000 mA (set limit to 500 mA if only using one labeling chamber) [varies from ~100 to 400 mA per active chamber, depending on what stage of the experiment is taking place]
- Reservoir A & B Temperatures = 25° C [20-30° C]
- Rotation = 0.01 Rpm (user-defined; 0.01 Rpm constant rotation speed is recommended)
- Stir bar spin RPM = 800 (user-defined; located in the password-protected menu)
- Polarity switching = 300 minutes (user-defined; 300 minutes is an efficient setting)

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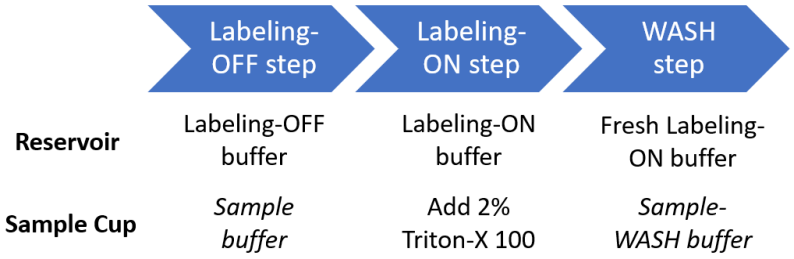
The recommended way to use SmartLabel is to perform the OFF-step on side A (left) and the ON- & wash-steps on side B (right), effectively designating each side of the device for distinct steps. As described below, washing the system between OFF- and ON-steps is key, as residual SDS can interfere with binding of labeling reagents to their targets during the ON-step. However, when SDS-containing Labeling-OFF solution is used only on side A, side B will only be exposed to SDS-lacking Labeling-ON solution, reducing risk of SDS contamination.

Similar to SWITCH experiments, labeling experiments with SmartLabel utilize different buffers for distinct phases of the labeling reaction. The Labeling-OFF and Labeling-ON buffers are what are loaded into SmartLabel's solution reservoirs located on top of the device at the front. The *Sample* & *Sample-WASH buffer*s are what are loaded into the sample cup along with the tissue sample and labeling reagents.



SmartLabel buffer volume by step: (mL)	Labeling-OFF	Labeling-ON	*Sample*	*Sample-WASH*
Pre-labeling incubation step	25			
Labeling-OFF step	475-500		1.5-4*	
Labeling-ON step		500		
WASH step		500		7-10*

Steps to running a labeling experiment with SmartLabel Pro:
This protocol has been optimized for labeling cleared samples the size of mouse brain hemispheres. If the user is interested in labeling brain regions exclusive of the olfactory bulb and cerebellum, it is recommended to remove these structures before performing experiments. This serves to help the sample fit better into the sample cup, reduce the number of antigenic sites, and bring the rostral-caudal length of the sample closer to its medial-lateral width and dorsal-ventral height, enhancing the efficiency of labeling via stochastic electrotransport.

The step lengths recommended below have been determined & optimized empirically and can be performed within a 48-hour period spanning 3 days (e.g., ~3:00 PM Monday to ~3:00 PM Wednesday).

1. Pre-labeling equilibration in Labeling-OFF buffer (*time: until sample sinks / overnight*)

Before labeling, samples must be equilibrated in ~25 mL Labeling-OFF buffer in a conical tube for overnight or until sinking (some sample types may not sink). Equilibration occurs quickest by using a shaker and by increasing temperature to up to 37° C.

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2. Labeling-OFF step (*time: 21 hours*)

After a sample has been equilibrated in Labeling-OFF buffer it is ready for labeling using SmartLabel. During the Labeling-OFF step, the labeling reagents are dispersed into the tissue under conditions in which their binding to target sites is prevented by the presence of SDS.

When reading the steps below, please keep in mind that it is best to design and prepare the labeling reagent cocktail solution in a small volume (~1 mL) of *Sample buffer* (step 2.d) before loading the sample into the sample cup, so that the sample does not start to dry in the cup if not fully submerged in liquid.

a. Determine which SmartLabel sample cup to use:
Depending on the size of your sample(s), determine which sample cup to use, either the small (~13 mm inner diameter) or the large (~19 mm). If compatible with your sample size, use the small cup whenever possible to conserve reagents and make it easier to position and stabilize the sample.

b. Pre-treat the sample cup with BSA-containing sample buffer:
During setup for each experiment, BSA-containing Sample buffer must be used to pre-treat the sample cup prior to the cup being exposed to any solution containing labeling reagents, in order to coat the cup's inner surface. *This is especially important when using antibodies, which can be adsorbed to the surface of the membrane, decreasing their effective concentration*.

   i. Always be careful when using spatulas, pipette tips, and any other instruments that may enter the sample cup. As long as the cup remains hydrated and is handled with care to prevent damage, it can be used indefinitely.
   ii. Retrieve the sample cup and corresponding sample cup insert from their storage in DI water with 10mM SDS + 0.02% sodium azide. Rinse the cup and insert under running DI water and then shake them dry.
   iii. Pipette 2 mL of fresh Sample buffer into the cup and then tilt & rotate the cup to coat its inner surface with solution. Allow it to incubate for several minutes before discarding the solution. Do not rinse the sample cup.

c. Load sample into insert & cup and find volume of *Sample buffer* needed to submerge sample:
In this step, add some Sample Buffer to the sample cup, load the sample cup insert and sample into the cup, and then determine how much liquid is needed to fully submerge the sample in the cup. **A mouse brain hemisphere, depending on its orientation and whether it has been trimmed as suggested above, will typically require ~2-4 mL of Sample buffer to be submerged in the small sample cup.**

   i. Pipette 1.5 mL of fresh Sample buffer into the sample cup, which is enough to fill the bottom compartment that contains the stir bar. If any air bubbles form in this compartment, remove them with a pipetter and then discard the tip. Then, load the sample cup insert into the cup to wet it.
   ii. Load the sample into the insert using a spatula and **ensure that the sample is positioned as close as possible to the bottom of the insert & cup.** Do this with the sample cup insert in your hand and held at an angle (either already in place in the cup or temporarily removed).
   iii. **Important: For samples whose width and height differ by more than ~25%, position the sample with its longest axis oriented vertically, i.e. perpendicular to the electric field. Labeling efficiency is greatly improved by orienting the sample such that its shortest dimension is parallel with the direction of the electric field, as shown below on the right (Orientation B).**

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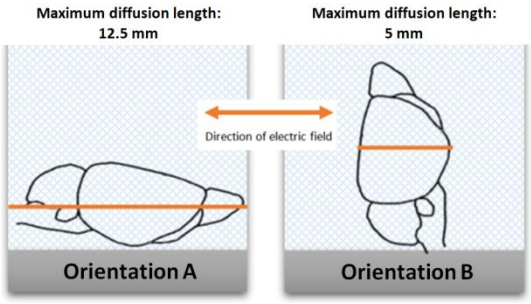

1. If you are labeling a few-millimeter -thick brain slice, it can be laid at an angle on the bottom of the insert such that the maximum diffusion length through the tissue is only slightly greater than it would be if the slice were positioned vertically and standing on a narrow edge. If positioning a slice this way, make sure that it will not move or fall flat during the experiment.

iv. Observe the solution level in the cup with respect to the sample and then add/remove solution as needed until the top of the solution column is ~1-2 mm above the top of the sample. (Do not put any removed solution back into the stock bottle.)

1. **A mouse brain hemisphere, depending on its orientation and whether it has been trimmed as suggested above, will typically require ~2-4 mL of Sample buffer to be submerged in the small sample cup**. **Record this volume and use it as the basis for calculating dilutions of labeling reagents in the next step.**

d. Prepare a cocktail of labeling reagents in Sample buffer, which will get added to the sample cup:
In this step, prepare a cocktail of labeling reagents in Sample buffer in a preparation tube, which then gets added to the sample cup. An immunolabeling experiment is described here as an example.

i. The final volume to keep in mind, for the purpose of determining reagent concentrations and dilutions, is that which was just calculated in step 2.c.iv.1 directly above. In practice, this is typically 2-4 mL when using the small sample cup.

ii. Because the reagent cocktail will be added to another volume of Sample buffer that is already present in the sample cup along with the sample, prepare the cocktail in a small volume of Sample Buffer, such as ~1 mL, but calculate concentrations with the larger, final volume in mind.

1. Due to how SmartLabel works in that labeling reagents are actively transported into the tissue by electrophoresis (rather than passively diffusing into the tissue from outside in), the absolute amount of antibody used (i.e., in µg) should be of primary concern vs. antibody concentration.
    a. Antibody amounts should be determined empirically but can initially be estimated by considering the abundance of antigenic sites in the tissue as well as tissue size/mass.
    b. For example, an antibody detecting the calcium-binding protein parvalbumin (PV), which is present in roughly 10% of neurons and is also highly-expressed in cerebellar Purkinje cells, is more abundant than tyrosine hydroxylase (TH), which is found in a relatively small number of neurons throughout the brain & brainstem. When using 1 µg/µl antibodies to label each of these proteins, 25 µg of anti-PV and 10 µg of anti-TH have been found to work well.
    c. Like with passive staining, however, use of too much antibody can result in higher levels of non-specific staining, so it is recommended to begin with a reasonable amount and consult with LifeCanvas regarding your results.

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iii. The current most widely-applicable antibody-labeling protocol involves using either:

1. Primary antibodies directly conjugated to fluorophores, or
2. **Including both primary antibodies (IgG) and secondary antibodies (either FC-specific F(ab) fragments (best) or generic F(ab) fragments and NOT full IgG secondaries) together during the Labeling-OFF dispersal step. The high concentration of SDS used during this step prevents the antibodies from binding to each other and to their targets.**
   - **Aim for a secondary-to-primary ratio of ~2:1, which should be calculated using the ratio of the antibody types' respective molecular weights** (an embedded Excel calculator is provided below), where:
     - MW = molecular weight (primary, 1° = 150 kDa; secondary, 2° = 50 kDa)
     - C = antibody concentration (if available, i.e. from manufacturer)
     - V = volume of antibody solution, calculated from '*C*' above and the *target weight (in µg) of 1° desired for the experiment, based on the mass of the tissue being labeled and your estimation of the number of antigenic sites present in the tissue (as described above)*

$$\text{molar ratio } \frac{1^\circ}{2^\circ} = \frac{1}{2} = \frac{\frac{V_{1^\circ} \cdot C_{1^\circ}}{MW_{1^\circ}}}{\frac{V_{2^\circ} \cdot C_{2^\circ}}{MW_{2^\circ}}}$$

$$V_{2^\circ} = V_{1^\circ} \cdot \frac{C_{1^\circ}}{C_{2^\circ}} \cdot \frac{MW_{2^\circ}}{MW_{1^\circ}} \cdot \frac{2}{1}$$

   a. Example, for a fully intact adult mouse brain hemisphere:
   Label #1 (e.g., PV, a marker of inhibitory interneurons):
      - C_{1° = 1 µg / µL (primary antibody concentration, from supplier)
      - C_{2° = 1.6 µg / µL (secondary antibody concentration, from supplier)
      - V_{1° = 25 µL (volume of primary antibody chosen to use)
      - V_{2° = 10.42 µL (volume of secondary antibody calculated to use)

   Label #2 (e.g., TH, a marker of catecholaminergic neurons):
      - C_{1° = 1.0 µg / µL
      - C_{2° = 1.6 µg / µL
      - V_{1° = 10 µL
      - V_{2° = 4.17 µL

Below is an embedded Excel calculator whose default values show a third example. Depending on if/how you have both Microsoft Word & Excel installed on your computer it may or may not be editable here within Word by double-clicking it repeatedly. If it is not editable, you can either (1) use the Excel file provided by LifeCanvas or (2) copy the table into Excel and enter the formula below the table into the last cell (to the right of V2), which will make the calculator functional.

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Secondary Antibody Volume Calculation formula		
Volume of Primary Antibody (in µL)	**V1**	10
Concentration of Primary Antibody (in mg/mL as indicated on the datasheet)	**C1**	1
Concentration of Secondary Antibody (in mg/mL as indicated on the datasheet)	**C2**	1.7
Molecular weight of Primary Antibody (in kDa)	**MW1**	150
Molecular weight of Secondary Antibody (in kDa)	**MW2**	50
Molar Ratio of Primary to Secondary	1	2
Volume of Secondary Antibody (in µL)	**V2**	3.921568627

Excel formula to solve for V2 is "=C2*C3*C6*C7/(C4*C5*B7)"

iv. Minimize introducing bubbles into the solution while adding components, and then mix the labeling reagent cocktail solution by simply inverting the tube a few times.

e. Add labeling reagent cocktail to sample cup, remove any bubbles, & ensure sample is immobile:
In this step you will add the labeling reagent cocktail to the sample cup. *Remember that the final liquid level in the cup should only be ~1mm above the top of the sample.* Therefore, if the sample is currently submerged in the cup, you should reduce the liquid level accordingly such that addition of the cocktail brings the final volume to the appropriate level. (In other words, you can always add fresh *Sample buffer* [without labeling reagents] to the cup to raise & top-off the final volume but avoid adding the cocktail to a too-full cup and then having to remove some, which causes unnecessarily dilution and is wasteful.)

i. Before loading the labeling reagent cocktail into the sample cup, use a pipetter to remove any bubbles present in the cup. It is especially important to remove a bubble that often forms in the lower stir bar compartment, which can be done by inserting a pipette tip through the grating on the floor of the cup.

ii. When loading the labeling reagent cocktail into the cup, do so slowly and with the tip of the pipette near the center of the cup and under the surface of the liquid. Gently mix the solution a few times by aspirating/ejecting it using a pipetter. Avoid creating bubbles and remove any that form.

iii. Make sure the sample is now fully submerged by the solution and add additional Sample buffer if needed.

iv. **Ensure that the sample cannot rotate or move on its own within the cup, such as can happen due to the vortex action of the stirrer.** If the sample is not fully secured in place, reposition it, and if needed lay thin strips of plastic mesh alongside it and/or on top of it to prevent it from moving during the experiment. In one configuration, the strips may form a U-shape when viewed from the side of the cup, such that the base of the U touches the top of the sample and the arms of the U are oriented vertically along the walls of the insert.

f. Dispense Labeling-OFF buffer into reservoir, put sample cup in chamber, & turn on electrophoresis:

i. Referring to the *Operation* section of the SmartLabel Pro User's Manual, load the reservoir to be used for the experiment (e.g., side A / left) with 475-500 mL of Labeling-OFF buffer.

ii. Place the loaded sample cup into the labeling chamber that corresponds to the reservoir that was loaded with Labeling-OFF buffer above (e.g., side A / left), gently ensuring that it is seated firmly in the hexagonally-shaped slot on the floor of the chamber.

1. The thin pieces of plastic forming the sample cup's outer wall can be fragile, so handle it with care and do not press down on it excessively. Make sure its base is properly aligned with the baseplate on the floor of the labeling chamber before attempting to push it in place.

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iii. On the SmartBox display, turn on the pump that serves the reservoir and labeling chamber in use (e.g., side A / left) and confirm flow of liquid into the labeling chamber.
   1. Start rotation of the sample cup, confirming it is operating correctly by going into the rotation menu and briefly increasing the speed so that rotation is readily apparent by eye (e.g., to ~2-3 Rpm), as described in the User's Manual. Set rotation speed to 0.01 Rpm for the experiment.
   2. Turn on the stirrer for the sample cup in use.
   3. Set/confirm side-A temperature as 25°C.
   4. Set the timer on the SmartBox display to 21 hours and start it counting.
      a. *Note that the concluded timer turns off sample cup rotation, which needs to be turned back on for subsequent steps.*

iv. Secure the lid to the labeling chamber and then to SmartLabel and turn on electrode power to the unit by pressing the lower-right button on the SmartBox display.

v. Confirm that current is being passed (typically ~125 - 200 mA per active chamber during the Labeling-OFF step, and rising a bit higher by the end), signaling that labeling is in progress.

3. Labeling-ON step (*time: 21 hours*)

Following the Labeling-OFF step, antibody/reagent concentration has been homogenized throughout the sample and probes are now close to high-affinity binding sites, but the presence of SDS has stopped them from binding to these sites. Adding Triton-X 100 to the sample cup inactivates SDS and allows binding to occur during the Labeling-ON step.

a. Turn off electrophoresis, remove sample cup from labeling chamber, & wash reservoir/chamber:
   i. Turn off the electrode power and open the lid to both SmartLabel and to the labeling chamber.
   ii. Turn off the pump serving the labeling chamber in use. Being careful that it may be slippery, **remove the sample cup from the chamber and place it on a bench top, being careful not to discard the solution that it contains**. The sample cup will remain sufficiently hydrated while sitting on the bench for ~15-20 minutes.
   iii. **Remember that the best way to perform SmartLabel labeling experiments is to carry out the OFF-step on side A (left) and the ON- & wash- steps on side B (right), so that SDS concentration can be kept as low as possible during the ON-step.** Nevertheless, take the opportunity while the side A surfaces are wet to wash this side of the device, while you are setting up for the ON-step to take place on side B.
   iv. To drain the buffers currently in place and to wash the system, refer to the *Maintenance - Buffer Change* section of the SmartLabel User's Manual. Use 500 mL of DI water for each of ~3 washes that should last ~5 minutes per wash, with some of the 500 mL being poured directly into the labeling chamber to provide a thorough cleaning.
      1. After the first wash (and as needed after subsequent washes), after the pump is turned off & the reservoir is emptied, use a squirt bottle of DI water to rinse the inner walls of the reservoir as well as the labeling chamber. Repeat until few to no bubbles result from pumping DI water through the system.

b. Add Triton-X 100 at a concentration of 2% to sample cup:
Add Triton-X 100 to the sample cup at a concentration of 2%, using [20% TX-100 in Labeling-ON buffer]. For example, if sample cup volume is 3 mL, add 300 µl of 20% TX-100 solution. This is the

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only step required to transition the sample cup to the Labeling-ON step -- the antibody-containing solution remains in place.

i. (Since during the Labeling-ON step a ~100% increase in sample cup volume typically occurs due to electro-osmotic flow across the sample cup's nanoporous membrane, an initial concentration of 2% TX-100 is targeted to account for this, so that the *final* concentration (following electro-osmotic flow -induced dilution) stays > ~1%.)

ii. **Note that some small amount of electro-osmotic flow can occur during the Labeling-OFF step, so observe solution height relative to sample top to judge the extent to which this may have occurred before determining how much Triton-X 100 to add.**

1. If large volume changes, up or down, occur during the Labeling-OFF step, please contact LifeCanvas for assistance with troubleshooting.

c. Dispense Labeling-ON buffer into reservoir, put sample cup in chamber, & turn on electrophoresis:

i. Fill the solution reservoir to be used for the experiment (e.g., side B / right) with 500 mL of Labeling-ON buffer.

ii. Place the sample cup into the corresponding labeling chamber (e.g., side B / right).

iii. As done when starting the Labeling-OFF step:

1. On the SmartBox display, turn on the corresponding pump, **start and confirm rotation of the sample cup and stirrer, and set the timer to 21 hours and start it counting.**
2. **Use of the timer is recommended especially for the Labeling-ON step, so as not to let the reaction proceed for longer than the recommended duration of 21 hours.**
3. Secure the lid to the labeling chamber & then to SmartLabel, set/confirm side-B temperature as 25°C, and turn on electrode power to the unit by pressing the lower-right button on the SmartBox display. Confirm that current is being passed.
4. **Note that over the course of the Labeling-ON step the volume of solution inside the sample cup often increases by ~100%. This is expected and accounted for in the design of the protocol.**

4. WASH step (*time: 6 hours*)

Following the Labeling-ON step, antibodies/reagents are now bound to their target sites, with relatively little free/unbound reagent left either in the sample cup solution or within the tissue. However, because experimental conditions vary and because different types of probes behave in unique ways, a WASH step is performed to improve signal-to-noise by removing free or weakly-bound probes from the tissue. In this WASH step, the sample is removed from the sample cup, which is then rinsed and filled with *sample-WASH buffer* before the sample is re-loaded and put back into the electric field. (This step may not be needed if using fluorescent nuclear dyes or antibody-fluorophore conjugates. Determine empirically.)

a. Turn off electrophoresis, remove sample cup from labeling chamber, & wash reservoir/chamber:
As done when ending the Labeling-OFF step:

i. Turn off the electrophoresis and open the lid to both SmartLabel and to the chamber.

ii. Turn off the pump serving the chamber in use and remove the cup from the chamber.

iii. Pour 250 mL of DI water into the solution reservoir and turn on the pump. Wash the system two times for a few minutes each and drain the reservoir when finished.

b. Remove sample from cup, rinse cup, pre-treat with *Sample-WASH buffer*, load with fresh buffer:

i. Use a spatula to remove the sample to a dish containing a few mL of *Sample-WASH buffer*.

ii. Remove the antibody solution from the sample cup, which you may wish to save rather than discard. While Triton-X's inhibition of SDS means that the solution can no longer be used

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effectively in SWITCH-based labeling experiments such as in SmartLabel, the solution may be useful in troubleshooting SmartLabel experiments that do not have a satisfactory outcome. For example, the antibody + Triton-X solution could be tested on thinner, ~100 µm thick tissue sections to validate whether the antibodies used to label intact tissue do indeed work in more traditional passive labeling applications.

iii. Gently rinse the sample cup and insert under DI water several times and shake dry.

iv. Pipette 2 mL of fresh *Sample-WASH buffer* into the cup and then tilt & rotate the cup to coat its inner surface with solution. Do this a couple of times and then allow it to incubate for a few minutes before discarding the solution. Do not rinse the cup.

v. Load fresh *Sample-WASH buffer* into the cup, being careful not to introduce any bubbles. Load the sample into the sample cup insert, immobilize the sample with mesh if needed, and place the insert into the cup. Add more *Sample-WASH buffer* until the height of the liquid column is ~2-3 mm from the top of the sample cup. If you are using the large cup, use ~10 mL of *Sample-WASH buffer* for this step.

c. Dispense Labeling-ON buffer into reservoir, put sample cup in chamber, & turn on electrophoresis:

i. Place the sample cup back into the labeling chamber on top of SmartLabel.

ii. Add 500 mL of Labeling-ON buffer to the reservoir in use.

iii. On the SmartBox display, turn on the corresponding pump, **start and confirm rotation of the sample cup and stirrer, and set the timer to 6 hours and start it counting.**

iv. Secure the lid to the labeling chamber & then to SmartLabel, set/confirm side-B temperature as 25°C, and turn on electrode power to the unit by pressing the lower-right button on the SmartBox display. Confirm that current is being passed.

5. Labeling experiment complete: Transfer sample to RI matching solution

Following the WASH step, the SmartLabel labeling protocol is complete. The sample can now be transferred to refractive index (RI) matching solution such as LifeCanvas's EasyIndex to incubate in preparation for imaging (time: several hours to overnight, depending on sample size).

a. Turn off electrophoresis, remove sample cup from labeling chamber, & wash reservoir/chamber:
As done following earlier steps:

i. Turn off the electrode power, open the lid to both SmartLabel and to the labeling chamber, and turn off the corresponding pump. Remove the sample cup from the chamber and place it on a bench top.

b. Remove sample from cup, transfer to RI matching solution:
Use a spatula to remove the sample to a small, clean container (e.g., cap of a 50 mL conical tube) holding a few mL of RI matching solution. Because many RI matching solutions are highly sensitive to change via dilution (or evaporation), the sample should first be incubated in these few mL for ~10-20 minutes, to allow time for the water-based *Sample buffer* to diffuse out of the sample.

i. It is equally important to avoid introducing dust and other physical contaminants to the RI matching solution as these may interfere with imaging.

ii. Transfer the sample to ~20-25 mL of fresh RI matching solution in a 50 mL conical tube, wrap the tube in aluminum foil, and place it on an orbital shaker for overnight at RT to 37° C.

c. Rinse cup and store it appropriately in 10 mM SDS with 0.02% sodium azide:
Gently rinse the sample cup with running water and then soak it for several minutes in a solution containing 10 mM SDS + 0.02% sodium azide to remove any labeling reagents that may still be present. Follow this with further rinsing of the sample cup under DI water and then store it in a sealed container submerged in 10 mM SDS + 0.02% sodium azide solution.