

QUICK REFERENCE CARD – TISSUE SAMPLES

Synlight-Rich™ Kit | Catalog No. SYN-RI0106 or SYN-RI0206; SYN-PU0206 (Verify only)


For use with the Microscoop® System

IMPORTANT

- Store kit at 2–8 °C.
 - Use light-controlled environment for photolabeling steps.
 - Prepare unlabeled (UL) and photolabeled (PL) samples in pairs.
 - DO NOT expose photolabeling reagents to light < 500 nm.
 - Use gloves, goggles, and protective clothing.
 - Do not use reagents past expiration date.
 - Biotin verification buffer is excited near 490 nm wavelength (e.g. FITC). If your ROI signal is within the wavelength range of 470-520 nm, please contact support@syncell.com.
-

REAGENT PREPARATION (sufficient for one UL/PL pair or 2 slides)


Reagent	Components
PBSTx	0.5% Triton-X-100 in PBS (5 mL Triton-X-100 in 99 mL)
Block 1 (A)	40 μ L Block 1 + 1960 μ L 0.5% PBSTx
Block 2 (B)	40 μ L Block 2 + 1960 μ L 0.5% PBSTx
Photolabel (C)	40 μ L Photolabel (C) + 1960 μ L PBS <i>100x dilution may be used for nuclear labeling</i>
Quench (D)	120 μ L Quench + 5.88 mL 0.5% PBSTx
Verify (G in Synlight-Rich Kit or P in Synpull Kit)	20 μ L Verify + 980 μ L 3% BSA in 0.5% PBSTx <i>For PL sample only</i>

 Vortex all reagents for 5 minutes at room temperature at 500 RPM and spin down briefly before preparation.

SAMPLE PREPARATION

Tissue: Prepare frozen (OCT-embedded) tissue sections or formalin-fixed, paraffin-embedded (FFPE) tissue sections with a thickness up to 20 μM for immunostaining. Multiple sections may be placed on a slide without overlapping.

All samples require fixation and permeabilization prior to Block.

 DO NOT use mounting media.

BLOCKING SAMPLES

1. Tissue samples must be **fixed, permeabilized and blocked** prior to adding Block 1 (see Sample Preparation).



For FFPE tissue sections, prior antigen retrieval is required to unmask epitopes.


2. Carefully **remove excessive moisture** from the glass slide and dry slide gently without disturbing the tissue.
 3. Use a **hydrophobic pen** to draw a rectangle **around the tissue perimeter**.
 4. Let the barrier **dry for 15 seconds**.
 5. Add **250 μL of prepared Block 1 Buffer** and incubate for **1 hour at room temperature**.
 6. Discard Block 1 Buffer and **wash with 250 μL of 0.5% PBSTx**.
 7. **Repeat Step 6** for two additional washes.
 8. Add **250 μL of prepared Block 2 Buffer** and incubate for **1 hour at room temperature**.
 9. Discard Block 2 Buffer and **wash with 250 μL of 0.5% PBSTx**.
 10. **Repeat Step 9** for two additional washes.
-

ROI STAINING


11. Stain with **primary antibodies** using optimal conditions.

 For samples with endogenous **fluorescently tagged proteins**, skip Steps 11-16.

12. Wash with **250 μ L of 0.5% PBSTx for 30 minutes at room temperature** on a rocker.
13. **Repeat Step 12** for two additional washes.
14. Stain with **secondary antibodies > 470 nm** (e.g., Alexa Fluor™ 568) using optimal conditions.
15. Wash with **250 μ L of 0.05% PBSTx for 30 minutes at room temperature** on a rocker.
16. **Repeat Step 15** for two additional washes.
17. Rinse with **250 μ L of PBS for a total of three times**.

 *Optional Stop Point: Store at 2–8 °C for up to 1 week in PBS containing 0.05% (w/v) Sodium Azide.*

PHOTOLABELING (LIGHT-CONTROLLED ENVIRONMENT)

18. Carefully **remove excessive moisture** from the glass slide of the UL sample and dry slide gently without disturbing the tissue.
19. Use a **hydrophobic pen** to re-draw a rectangle **around the tissue perimeter** if necessary. Let the barrier **dry for 15 seconds**.
20. **Affix a 0.05 mm thick double-sided tape** ([iSpacer®](#), [cat IS201/IS211](#), [SUNJin Lab](#)) around the tissue perimeter.
21. Add **250 μ L of prepared Labeling Reagent** to the sample.
22. Overlay a microscope **no. 1.5 cover glass** on the sample.
-  Ensure there are no bubbles over the tissue section.
23. Secure the cover glass by **sealing the perimeter** with the double-sided tape by gently pushing the cover glass down on the spacer tape.
24. Repeat **Steps 18-23 for the PL sample**.

25. Place the **PL sample cover glass side down** on the **Microscoop® stage**.
26. Place the **UL sample alongside the PL sample** on the Microscoop® stage for environmental consistency.
27. **Run the Microscoop® to photolabel the PL sample** in accordance with the Autoscoop® Software User Manual and conditions specified by your FAS.

⚠ DO NOT use DAPI (365 nm) or brightfield imaging when sample are immersed in Labeling Reagent.

28. Leave samples on the instrument until **photolabeling is complete** (time varies).


⚠ DO NOT leave samples overnight. Monitor photolabeling progress to ensure the photolabeling reagent is not drying out.

29. Carefully **remove cover glass and double-sided tape**.

30. Discard Labeling Reagent and wash with **250 μ L of prepared Quench for 5 minutes at room temperature** on a rocker.

31. **Repeat Step 30** for two additional washes.


32. **Rinse with PBS** and **store at 2–8 °C** until ready to proceed to the Synpull.

 *Optional Stop Point: If pooling samples prior to Synpull, repeat Steps 19-33 as needed. Samples may be stored for up to 1 month at 2-8 °C. Store in PBS containing 0.05% (w/v) Sodium Azide and keep in the dark.*

PHOTOLABELING VERIFICATION

33. **Aliquot 120 μ L of Scrape (A)** into clean and labeled 1.5 mL tube for the UL sample and a second tube for the PL sample to prevent cross-contamination.
34. **Rinse the UL sample** with **250 μ L of ultrapure water**, then gently discard.
35. **Scrape cells** according to the Synpull IFU by adding **40 μ L of Scrape (A) to the UL sample**, leaving a small area behind for verification.
36. Mechanically scrape cells from the UL slide **and transfer to a clean and labeled 1.5 mL tube** using a pipette.
37. **Repeat Steps 35-36 two more times** by adding an additional **40 μ L of Scrape (A) to the UL sample** for a total of 120 μ L.

38. Perform **Steps 34-37 with the PL sample.**

 *Optional Stop Point: Store scraped samples at 2–8 °C for up to 24 hours until ready to perform the Synpull protocol. Long term, samples may be stored at -20 °C or -80 °C for up to ??*

39. Add **250 µL of prepared Verification Buffer** to the PL sample.


40. **Incubate in the dark for 3 hours** at room temperature while rocking.

41. Discard Verification Buffer and **wash with 0.5% PBSTx** for a total of **three times**.

42. **Rinse three times** with PBS.

43. **Image using Microscoop®** FITC channel (490 nm) using the following parameters: 10x/0.45 Dry Objective, 800x800 resolution, 10% lamp intensity, 1000 ms exposure time.

 **S/N Ratio must be > 8** (see Synlight-Rich IFU for quantifying labeling efficiency).

 Some tissue samples may appear to have significant background. If samples do not meet this criterion, please contact your FAS or support@syncell.com for assistance.

44. Proceed with **Step 7 in Section 7.2.2. of the Synpull 2.0 protocol** to continue harvesting samples.