

QUICK REFERENCE CARD – CELL 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.
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REAGENT PREPARATION (sufficient for one UL/PL pair or 2 slides)

Reagent	Components
PBSTx	0.1% Triton-X-100 in PBS (1 mL Triton-X-100 in 99 mL
Block 1 (A)	40 μ L Block 1 + 1960 μ L 0.1% PBSTx
Block 2 (B)	40 μ L Block 2 + 1960 μ L 0.1% 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 0.1% PBSTx
Verify (G in Synlight-Rich Kit or P in Synpull Kit)	20 μ L Verify + 980 μ L 3% BSA in 0.1% 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

Cells: Seed 2.4×10^5 cells/well on single-well slides or as needed to achieve 80-90% confluency in [one-well chambered cover glass slides](#).

All samples require fixation with paraformaldehyde (PFA), acetone, or methanol and permeabilization prior to Block.



If ROI targets are at the edges of cells or synapses, decrease seeding density to allow for appropriate masking of targets.

BLOCKING SAMPLES

1. Cells must be **fixed and permeabilized** prior to adding Block 1 (see Sample Preparation).
2. Add **1 mL of prepared Block 1 Buffer** and incubate for **30 min at room temperature**.
3. Discard Block 1 Buffer and **wash with 1 mL of 0.1% PBSTx**.
4. **Repeat Step 3** for two additional washes.
5. Add **1 mL of prepared Block 2 Buffer** and incubate for **15 min at room temperature**.
6. Discard Block 2 Buffer and **wash with 1 mL of 0.1% PBSTx**.
7. **Repeat Step 6** for two additional washes.



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

ROI STAINING

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




For samples with endogenous **fluorescently tagged proteins**, skip Steps 8-13.

9. Wash with **1 mL of 0.1% PBSTx for 10 minutes at room temperature** on a rocker.
10. **Repeat Step 9** for two additional washes.

11. Stain with **secondary antibodies > 470 nm** (e.g., Alexa Fluor™ 568) using optimal conditions.
 12. Wash with **1 mL of 0.1% PBSTx for 10 minutes at room temperature** on a rocker.
 13. **Repeat Step 12** for two additional washes.
 14. Rinse with **1 mL of PBS for a total of three times**.
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PHOTOLABELING (LIGHT-CONTROLLED ENVIRONMENT)

15. Add **1 mL of prepared Labeling Reagent** to each of the paired UL and PL samples.
 16. Place the **PL sample** on the **Microscoop® stage**.
 17. Place the **UL sample alongside the PL sample** on the Microscoop® stage for environmental consistency.
 18. **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 is immersed in Labeling Reagent.**
19. Leave samples on the instrument until **photolabeling is complete** (time varies).
 20. Discard Labeling Reagent and wash with **1 mL of prepared Quench for 5 minutes at room temperature** on a rocker.
 21. **Repeat Step 20** for two additional washes.
 22. **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 15-22 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

23. **Aliquot 120 µL 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.

24. **Rinse the UL sample** with **1 mL of ultrapure water**, then gently discard.
 25. **Scrape cells** according to the Synpull IFU by adding **60 μ L of Scrape (A) to the UL sample**, leaving a small area behind for verification.
 26. Mechanically scrape cells from the UL slide **and transfer to a clean and labeled 1.5 mL tube** using a pipette.
 27. **Repeat Steps 25-26** one more time by adding an additional **60 μ L of Scrape (A) to the UL sample** for a total of 120 μ L.
 28. Perform **Steps 24-27 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 ??*
29. Add **1 mL of prepared Verification Buffer** to the PL sample.
 30. **Incubate in the dark for 1 hour** at room temperature while rocking.
 31. Discard Verification Buffer and **wash with 0.1% PBSTx** for a total of **three times**.
 32. **Rinse three times** with PBS.
 33. **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). If samples do not meet this criterion, please contact your FAS or support@syncell.com for assistance.
34. Proceed with **Step 7 in Section 7.2.2. of the Synpull 2.0 protocol** to continue harvesting samples.