

WORKFLOW QUALITY CHECKLIST

Please complete this form while performing the protocol. If you answer "No" to any questions or encounter any issues, contact your Syncell representative.

Sample type: _____ Replicate: _____ of _____

No. of cells seeded per one-well chamber (if applicable): _____

Tissue section thickness (if applicable): _____

Number of slides or tissue sections to be photolabeled: _____

PHOTOLABELING ASSESSMENT

Imaging resolution (800x800 or 1600x1600): _____ Objective used: _____

Record a representative picture to assess signal quality and mask:

IMMUNOSTAINED IMAGE	GENERATED MASK

Record the following information after labeling is completed according to the log file:

Total Pixel Count (at least 10^7):		Z-offset:	
Labelling Power: _____ mW	Labeling Time: _____ μ s	Total Run Time: _____	

Record 3 FOVs as an example of photolabeling efficiency measurement.

S: _____, S/N ratio: _____	S: _____, S/N ratio: _____	S: _____, S/N ratio: _____
----------------------------	----------------------------	----------------------------

For the best verification of labeling, we also recommend the use of an inverted confocal microscope system. If multiple slides are pooled, record this information for each slide.

- Confirm that the labelling signal is localized in the target:
- Confirm that the S/N ratio > 8:

CELLS SCRAPING and LYSIS

- Confirm using a microscope that cells are scraped:
- Confirm efficient lysis. For fixed cells: no visible precipitate after sonication. For tissue samples, low precipitates (acceptable based on experience):

TOTAL PROTEIN MEASUREMENTS

- Record Total Protein quantification for UL:
- Record Total Protein quantification for PL:

AFFINITY PURIFICATION and ON-BEADS DIGESTION

- Confirm that equal quantities of proteins from PL and UL are transferred to Proteomics tubes:
- Record the total volume: PL UL
- Confirm pulldown input-to-volume ratio (100 µg per 800 µL):
- Record the µL of beads used:
- After washing and sonicating. Confirm the appearance of beads as shown in Figure 7.3:
- Confirm Digest (K) stored at -20 C and freeze-thawed less than 3 times.
- Confirm beads well mixed during digestion (ELMI Intelli-Mixer uu-mode-60):

LC-MS/MS and DATA ANALYSIS

- Confirm Proteomics tubes are used for on-bead digestion.
- Confirm an on-line C18 trapping column or off-line desalting procedure by ZipTip with C18 resin is used.
- **Analysis of the Chromatogram.**
Confirm that distinct peaks are present and evenly distributed.
- **Report total number of proteins identified:**
- **Report PL/ UL enriched protein number:**
When running a Positive Control, confirm % nuclear proteins is > 80 (DDA):
- **Analysis of the Volcano Plot**
Confirm that the data distribution looks asymmetrical and right-shifted.