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Metabolic Labeling with AHA or HPG

General Information

For imaging, culture cells on cover slips or other desired imaging-compatible surface, approximately 1×10^6 cells are sufficient for one detection. 1×10^6 cells are required to prepare 100 µg of lysate.

Metabolic Labeling of Cells

The following is a standard protocol for preparation of AHA or HPG labeled cell lysates and conditioned media. However, investigators may wish to determine the optimal concentration of metabolic reagent as well as labeling time individually for metabolic labeling for each cell type on a small-scale first.

1.1 Prepare a stock solution of AHA or HPG metabolic labeling reagent with DMSO to make a 500X or 1,000X stock solution. This process ensures that the DMSO concentration is not more than 0.1–0.2% in the cell culture. Aliquot and store any unused reagent at –20°C. When stored as directed, this stock solution is stable for up to 1 year..

1.2 Wash cells once with warm PBS or HEPES, add methionine-free medium to the cells and incubate the cells at 37°C for 30–60 minutes to deplete methionine reserves.

1.3 Add the desired concentration of metabolic labeling reagent to the cell medium, gently mix, and incubate at 37°C, 5% CO_2 for 1-4 hours. Working concentrations vary from 10 µM to 100 µM. Working concentrations below or above this range are also possible, and should be optimized for metabolic labeling for each cell type.

1.4 The cells are ready for imaging, flow analysis, or harvesting..

Cell Preparation for Imaging or Flow Cytometry.

2.1 Wash the cells with PBS or HEPES to remove excess of metabolic labeling reagent.

2.2 Fix the cells with 4% paraformaldehyde in PBS for 15 minutes.

2.3 Permeabilize the cells using a fixation and permeabilization protocol of your choice. Click reaction is compatible with all protocols.

Example: 0.25% Triton® X-100 in PBS, 15 minutes or
1% BSA/0.1% saponin in PBS, 15 minutes.

2.4 Wash the cells with 3% BSA in PBS.

2.5 The cells are now ready for the click reaction with the corresponding azide- or alkyne- tagged reagent using Click-&-Go™ Cell Reaction Buffer Kit

Harvesting Suspension Cells

3.1 Pellet the cells by centrifugation at 400 × g for 5 minutes. Discard the supernatant.

3.2 Resuspend the cell pellet in PBS, gently pipette the cells up and down using 5 mL PBS for cells from a 100 mm dish or 1 mL PBS per well for cells from a 6-well plate

3.3 Pellet the cells by centrifugation at 400 × g for 5 minutes. Discard the supernatant.

3.4 Repeat the PBS wash 2 more times for a total of 3 washes to remove serum.

3.5 Pellet the cells by centrifugation at 400 × g for 5 minutes. Discard the supernatant. The cell pellet can be used directly in step or flash frozen and stored at –80°C until use.

3.6 Proceed to the cells lysing step.

Harvesting Adherent Cells

Note: If analyzing cell surface proteins, do not use trypsin to detach cells, because trypsin cleaves cell surface proteins. You can lyse the cells directly in the culture dish, or, if desired, use a non-enzymatic dissociation buffer or a cell scraper, and pellet the cells. You can use the cell pellet directly in next step or flash freeze and store it at –80°C until use.

4.1 Remove the medium and wash the cells three times with PBS.

4.2 Proceed immediately to the cells lysing step.

Harvesting Suspension Cells

Do not use DTT, TCEP, or β-mercaptoethanol because they will reduce the azide**.**

5.1 Prepare the lysis buffer by adding protease and phosphatase inhibitors at appropriate concentrations to 1% SDS in 50 mM Tris-HCl, pH 8.0. You need 500 µL lysis buffer per 100 mm dish and 200 µL lysis buffer per well of a 6-well plate

Note: Protease and phosphatase inhibitors are optional but recommended to ensure sample integrity.

If a probe sonicator is not available, add 250 U of Benzonase® endonuclease per mL of lysis buffer.

5.2 For adherent cells, add 500 µL lysis buffer per 100 mm plate or 200 µL lysis buffer per well of a 6-well plate to the labeled cells. If adding the lysis buffer directly to the plate, tap or rotate the plates so the lysis buffer covers the bottom surface of the plate.

For suspension cell pellet, add 50 µL lysis buffer per 1×10^6 cells.

5.3 Incubate the cells for 15–30 minutes on ice, then tilt the plates and pipet the lysate into a 1.5 mL microcentrifuge tube. If the lysis buffer does not contain Benzonase® endonuclease, the lysate may be very viscous due to the DNA from the lysed cells.

If using Benzonase® endonuclease, proceed to step 5.5

5.4 Sonicate the lysate with a probe sonicator to solubilize the proteins and disperse the DNA.

5.5 Vortex the lysate for 5 minutes.

5.6 Centrifuge the cell lysate at 13,000–18,000 × g at 4°C for 5 minutes.

5.7 Transfer the supernatant to a clean tube and determine the protein concentration using BCA assay or another method.

5.8 The protein sample is now ready for reaction with an azide or alkyne detection molecule. If not used immediately, store the sample at –20°C for up to 2 weeks. If you wish to store the sample for more than 2 weeks, precipitate the sample.

References

Dieterich, D. C., *et al*, (2006). Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). *Proc Natl Acad Sci* **103**, 9482.

Weslee S.G., *et al*, (2017). Bioorthogonal Noncanonical Amino Acid Tagging (BONCAT) Enables Time-Resolved Analysis of Protein Synthesis in Native Plant Tissue. *Plant Physiol.,* **173,** 1543–53.

Hatzenpichler R., *et al*, (2014). In situ visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry. *Environ Microbiol.* **16** 2568-90.