Assaying Cell Proliferation and DNA Replication with Click Chemistry

Cell proliferation assays are widely used in cytotoxicity studies, cancer research, and many other areas of cell biology. Many of them allow visualization of proliferating cells by fluorescent labeling and are suitable for high-throughput screening.

Detection of replicated DNA is arguably the most direct way to detect proliferation. This has been accomplished using bromodeoxyuridine (BrdU) nucleoside, which incorporates into DNA during replication. Then, these nucleoside modifications in cellular DNA can be detected by anti-BrdU antibodies. Despite its specificity, this assay is tedious and difficult to reproduce because the treatment of cells with harsh reagents is required to expose DNA to antibodies that otherwise do not penetrate cellular structures.

A milder alternative is ethynyl deoxyuridine (EdU), which can be delivered to cells by adding it to a culture medium or injecting it into an animal. Following its incorporation into DNA, this nucleoside can be conjugated with various fluorescent dye azides under copper(I) catalysis, which provides fluorescent staining of the replicated DNA. The assay is easy to perform, it is fast and reproducible. It does not require harsh treatment of cells (only permeabilization with Triton is needed) and, therefore, better preserves cellular structures. After the treatment with a dye azide and the last washing step (step 8), the cells can be labeled using fluorophores with various emission wavelengths, such as Hoechst, DAPI, or fluorescently labeled antibodies.

Required reagents

- 5-Ethynyl-2'-deoxyuridine (EdU)
- Copper(II)-BTTAA complex — click chemistry catalyst
- Ascorbic acid — reductant for copper
- Fluorescent dye azides
- DMSO, labeling grade — solvent for azides
- Triton X-100 (or Tween-20)
- PBS, pH 7.4
- 100 mM Tris buffer, pH 7.4

Protocol

The exact protocol depends on a particular cell or tissue type, but the general workflow is described below:

1. Incubate cells/tissue with 10–20 μM EdU for the desired time.
2. Fix cells with 3.7% formaldehyde in PBS for 15 min.
3. Wash cells with PBS.
4. Permeabilize cells with PBS containing 0.2% Triton X-100 (or 0.5% Tween-20) for 30 min.
5. Wash cells again with PBS.

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6. Prepare a mixture containing 2 mM copper(II)-BTTAA complex, 10 mM ascorbic acid, and 5 µM dye azide in 100 mM Tris buffer, pH 7.4 (for sulfonated azides) or 100 mM Tris buffer, pH 7.4 containing 50% DMSO (for non-sulfonated azides). This mixture should be freshly prepared because Copper(I) is not stable in aqueous solutions.
7. Incubate cells with the click reaction mixture for 30 min.
8. Wash cells with PBS.
9. *(Optional)* If cells have to be labeled with a different fluorophore, proceed with staining using your standard protocol.

Sulfonated (water-soluble) azides, such as sulfo-Cyanine azides and AF azides, produce the best results. When using non-sulfonated azides, such as Cyanine azides, or BDP azides, ensure 50% DMSO concentration in the reaction mixture.

References

1. *Ranall, M.; Gabrielli, B.; Gonda, T.* Adaptation and validation of DNA synthesis detection by fluorescent dye derivatization for high-throughput screening. *BioTechniques*, **2010**, 48(5), 379-386. doi: 10.2144/000113410

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