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EdU Staining Protocol for *Sulfolobus acidocaldarius*

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Abstract

This protocol describes how to stain EdU after its incorporation into Sulfolobus acidocaldarius. EdU incorporation allows visualization of newly synthesized DNA, making it a useful method for studying the cell cycle. The staining process involves an EdU Click-It reaction using Alexa Fluor azide and subsequent washing steps before analysis by microscopy or flow cytometry. Compared to other DNA labeling techniques, this protocol offers high specificity and ease of implementation.

Related publication(s): Gristwood et al. The sub-cellular localization of Sulfolobus DNA replication. *Nucleic Acids Res*. 2012 Jul;40(12):5487-96. doi:10.1093/nar/gks217.

Background

Gristwood et al. (2012) developed an EdU-based labelling method to study DNA replication in Sulfolobus, leveraging the thymidine salvage pathway to incorporate base analogues into newly synthesized DNA. This approach enables high-resolution visualization of replication dynamics at the single-cell level.

Unlike traditional methods such as neutral–neutral 2D gel electrophoresis and marker frequency analysis (MFA), which provide population-level insights into replication progression, EdU incorporation allows for direct imaging of replication sites. EdU, a thymidine analogue, is phosphorylated by thymidine kinase (TK) and incorporated into DNA. Detection is achieved through copper-catalyzed click chemistry, in which an azide-linked fluorophore reacts with EdU's alkyne group, eliminating the need for DNA denaturation required in halogenated thymidine analogue (e.g., BrdU) detection.

Using this technique, Gristwood et al. (2012) demonstrated that replisomes originating from the three replication origins in Sulfolobus do not form a single replication cluster. Instead, sister replisomes from the same origin tend

to remain co-associated, with a notable localization of replication foci near the cell periphery. This method provides a valuable tool for studying archaeal DNA replication with spatial precision.

Materials

Product name	Brand	Manufacturer	Catalogue number	Notes
EdU stock (0.2M)	Thermo Fisher	C10350	Store at -20°C	EdU stock (0.2M)
Click-iT reaction buffer	Thermo Fisher	C10269	Keep at 4°C	Click-iT reaction buffer
CuSO4	Sigma-Aldrich	209198	Protect from moisture	CuSO4
Alexa Fluor azide	Thermo Fisher	A10266	Light-sensitive	Alexa Fluor azide

Equipment

Equipment name	Brand	Manufacturer	Catalogue number	Notes
Pipettes and tips	Gilson	Various	For precise liquid handling	Pipettes and tips
Microcentrifuge tubes	Eppendorf	Various	1.5 mL for reaction mixtures	Microcentrifuge tubes
Flow cytometer	BD Biosciences	Various	For cell analysis	Flow cytometer
Fluorescence microscope	Zeiss/Olympus	Various	For visualization of stained cells	Fluorescence microscope

Protocol

Step 1 EdU Incorporation and Fixation

1. Thaw EdU stock and add 10 µL of 0.2M EdU per 10 mL of exponentially growing S. acidocaldarius culture.
2. Incubate cells at 75°C for the desired duration according to experimental design.
3. Fix cells using ethanol fixation (see Ethanol Fixation Protocol). Samples can be stored for up to one week at 4°C.

Step 2 EdU Labeling

1. Rehydrate the fixed cells with PBS-TA (PBS with 0.2% Tween-20 and 1% BSA).
2. Prepare the EdU Click-It reaction mixture:
    - 860 µL Click-It reaction buffer
    - 40 µL CuSO4
    - 2.4 µL Alexa Fluor azide
    - 100 µL reaction buffer additives (prepare fresh, 1:10 dilution in ddH2O)

3. Incubate the sample with 50 µL of Click-It cocktail per sample for 1 hour at room temperature, protected from light.
4. Wash the sample twice with PBS-TA.
5. Counterstain the sample with Hoechst or Syto13 for nuclear staining.
6. Prepare for flow cytometry or fluorescence microscopy.

Downstream analysis

Cells can be analyzed using flow cytometry or fluorescence microscopy to assess EdU incorporation and DNA content. Representative histograms or images should be included to show results.

Recipes

Recipes

PBS-TA Preparation:

- 0.2% Tween-20
- 1% BSA
- PBS (1X) Prepare fresh before use.

Additional Notes

- Ensure all reagents are properly stored and protected from light where applicable.
- The Click-It reaction chemistry is highly specific but requires careful reagent handling to avoid precipitation.
- Maintain sterility where necessary to prevent contamination.

Competing interests

The authors declare that they have no competing interests.

References

1. Gristwood, T., Duggin, I.G., Wagner, M., Albers, S.V. and Bell, S.D. (2012) 'The sub-cellular localization of S*ulfolobus* DNA replication', *Nucleic Acids Research*, 40(12), pp. 5487–5496. doi:10.1093/nar/gks217.