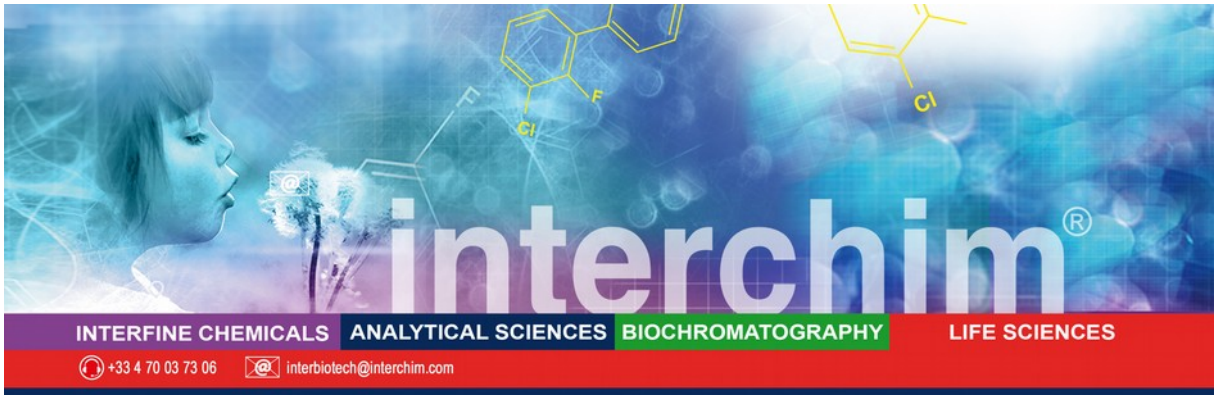
FT-MM982A

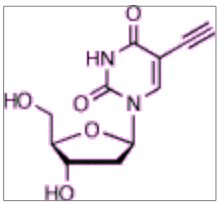


EdU, Cell Proliferation Assay

Cell proliferation assay with "click" chemistry as an alternative to ^{3}H-thymidine and BrdU methods

Product Description

Name :	**5-ethynyl-2'-deoxyuridine (EdU)** Cell proliferation assay based on click chemistry
Catalog Number :	FP-MM9829, 25 mg FP-MM982B, 100 mg FP-MM982C, 500 mg
Structure :	$C_{11}H_{12}N_2O_5$
Molecular Weight :	MW= 252,22
CAS No :	61135-33-9
Solubility:	DMSO



Storage: +4°C (long term at -20°C) Protect from light and moisture

Introduction

Click method is a method for labeling DNA *in vivo* that allow to image the replicated DNA in the context of well preserved cellular and chromatin ultrastructure. 5-ethynyl-2′-deoxyuridine (EdU) is readily incorporated into cellular DNA during DNA replication. The terminal alkyne group is then detected through its reaction with fluorescent azides, in a Cu(I)-catalyzed [3 + 2] cycloaddition ("click" chemistry). This method is highly sensitive and much faster than BrdU detection. In addition, because the reagents are almost 1/500th the size of an antibody molecule, they have a much higher diffusion rate and penetrate the tissue much more effectively, which allows the rapid, whole-mount stain of large tissue and organ fragments. Finally, the reaction between ethynyl groups on DNA and fluorescent azides does not require denaturation of the specimen; this allows good structural preservation (Salic, 2008).

Directions for use

Guidelines for use (Salic, 2008)

- Grow cells on glass coverslips in DMEM supplemented with 10% adapted serum, penicillin, and streptomycin.
- Add EdU to the culture media in concentrations ranging from 10 nM to 10 µM, for durations of time between 1 and 24 h
- After labeling, wash cells two to three times with PBS followed by addition of normal tissue culture media.

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Note: If cells are to be analyzed immediately after labeling, the washes can be omitted. Instead permeabilize and fixe cells.

- Fix cells by using a standard formaldehyde fixation protocol. The fixed cells can be stored at 4°C and stained months later without loss of signal.
- After formaldehyde fixation, rinse cells once with TBS (although formaldehyde does not interfere with the detection reaction)
- Stain by incubating for 10–30 min with 100 mM Tris (from 2M stock, pH 8.5), 0.5–1 mM CuSO4, 1–100 µM fluorescent azide (from 10 to 100 mM stocks in DMSO), and 50–100 mM ascorbic acid (added last to the mix from a 0.5 M stock in water). The staining mix has to be prepared fresh each time and to be used for staining cells immediately after addition of ascorbate.
- After staining, wash the cells on coverslips several times with TBS with 0.5% Triton X-100.
- EdU-stained cells can be immunostained by using standard protocols.
- Counterstained cells with Hoechst or DAPI, mount in standard mounting media and image by fluorescence microscopy. The EdU stain is stable indefinitely at 4°C or lower temperatures.
  For high-throughput screening, we recommand the protocol from Ranall M. *et al.* (2010).
  Other protocol may be found in the literature.

References

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Technical and scientific information

Related products

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- FluoProbes 550A azide (554/576), FP-FI2090
- FluoProbes 565A azide (563/592), FP-YE4990
- FluoProbes 590A azide (594/624), FP-YE5000
- FluoProbes 633A azide (629/657), FP-YE5010
- FluoProbes 647N azide (644/669), FP-YE5020
- FluoProbes 655A azide (663/684), FP-YE5030
- $CY_{anine}3$ azide, FP-EV0900
- $CY_{anine}5$ azide, FP-EV0910
- FAM azide, 5-isomer, FP-EV0920
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- ROX azide, 5-isomer, FP-EV0950
- ROX azide, 6-isomer, FP-EV0960
- TAMRA azide, 5-isomer, FP-EV0880
- DMSO anhydraous, FP-JW7390
- CuSO4 5H2O, 13495A
- Hoechst 33342, FP-BB1340
- RedDot 2 for nucleus-specific counterstaining of fixed cells and tissues, HO8720
- PBS powder, 68723A
- Fluoro-Gel mounting medium with DAPI, DT094A
- 5-ethynyl dUTP, solution 100mM, DQI622

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- azide-PEG4-dUTP, DQI711

Ordering information

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Please inquire for higher quantities (availability, shipment conditions).

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