Detection of DNA replication by 5-ethynyl-2'-deoxyuridine

Protocol for flow cytometry

Stock solutions to be prepared:

Solution A **- 8 mM $CuSO_4$ and 200 mM NaCl**.

Dissolve $CuSO_4$ in deionized water, add an appropriate volume of 1 M NaCl (stock solution) and fill with deionized water to the final volume. Store at 4°C.

Solution B **- 20 mM sodium ascorbate and 40 mM glycine**.

Dissolve sodium ascorbate and glycine in deionized water and fill with deionized water to the final volume. Store at −20°C.

10 0M azide dye solution (e.g. FAM azide)

Dissolve azide dye in DMSO. Store at −20°C.

**As an aqueous solution of sodium ascorbate is the subject of quick air oxidation, prepare the aliquots in Eppendorf tubes and seal them with parafilm.*

Protocol

1. Incubate the cells with 10 µM 5-ethynyl-2'-deoxyuridine (EdU) for the required time.
2. Centrifuge the samples (500× g, 5 min)
3. Discard the supernatant and add 1× PBS (10 ml if 15 ml tubes are used).
4. Centrifuge the samples (500× g, 5 min).
5. Discard the supernatant and add 3 ml 1× PBS. Mix by pipetting.
6. Shake the tube and slowly drop 7 ml of 100% ethanol pre-cooled to -20°C during the shaking. Put the samples into the freezer and incubate at least for 60 min.

**It is possible to leave the samples in the freezer for several weeks without loss of the signal. A shorter time did not result in a decrease of the signal, however it can result in the gradual loss of cells.*

7. Centrifuge the samples (500× g, 5 min).
8. Prepare fresh EdU labelling solution from stock solutions consisting of copper(I) solution with azide dye (the final concentration of azide dye is 10 µM): Add one volume of the solution A, 1/500 of volume of azide dye and at last, add one volume of the solution B. Mix on vortex for 5 s. Prepare the mixture just before labelling.

**Pre-warm solution A and solution B to 25°C before use.*

9. Discard the supernatant and add 150 – 200 µl of the prepared EdU labelling mixture and incubate for 30 minutes at room temperature. During incubation gently and regularly shake the tube, do not vortex (three shaking steps are enough).
10. Add 3 ml of 1× PBS, mix by pipetting.
11. Centrifuge the samples (500× g, 10 min).
12. Discard the supernatant and add 3 ml of 1x PBS.

**If RNA has to be removed (e.g. if propidium iodide, is used for DNA labelling), centrifuge the samples, discard supernatant, add 250 µl of 1× PBS with RNase A (100 µg/ml) and incubate for 30 minutes at 37°C). During incubation gently and regularly shake the tube, do not vortex (three shaking steps are enough). Then, add 3 ml of 1× PBS.*

13. Centrifuge the samples (500× g, 10 min).
14. Discard the supernatant and add 200 µl of DAPI (final concentration: 1 - 10 µM) in 25 mM Tris–HCl, pH 7.5 and 150 mM NaCl or propidium iodide (10 µg/ml) in 1× PBS, mix by pipetting and incubate for 10 to 30 minutes at room temperature. During incubation gently and regularly shake the tube, do not vortex (three shaking steps are enough).