EdU labeling and Staining Protocol.v2

This is a protocol provided by Shiqi from Dr. Shihuan Kuang's laboratory based on the paper published in PNAS http://www.pnas.org/content/105/7/2415.long

Labeling protocol

1. Dissolve EdU (Carbosynth, Cat#: NE08701, $380 for 2 g) in saline at 1 mg/ml and store at -20°C. (Note: It costs about $3000 if order from ThermoFisher Cat# A10044 or E10187)
2. Inject 0.2 ml for each mouse every 24 hrs, or 0.1 ml every 12 hrs.
3. Incubate cells at 1 μM in culture medium for cultured cells.

Staining protocol

1. Fix the cells or sections if needed. Unfix cells or section could also be stained.
2. Incubate the cells with the staining solution for 10-20 min in the dark. For sections, stain for 30 min.
3. Wash the cell or sections 3 x 5 min or longer using PBS. Washing is very important especially for the sections to avoid strong background. Cells can't be cultured after staining even though they can be stained alive.
4. Do not expose the stained cells or sections in the light for long time.
5. Immunofluorescence should be done after EdU staining. Cu(I), the catalyst of the EdU staining, could destroy GFP. If cell GFP detestation is needed, you can detect GFP using antiGFP antibody after EdU staining.

Staining solution preparation

Tris 100 mM (from 2 M stock pH 8.5)
CuSO4 1mM
TAMRA (fluorescent azide, Invitrogen, cat# T10182, $353 for 0.5 mg) 10 μM (from 10 mM stocks in DMSO (Dissolve 0.5 mg in 90 μl of DMSO)
Ascorbic acid 100 mM (added last to the mix from a 0.5 M stock in water)
PBS balance to 1 ml

Note: The staining solution should be made fresh each time. Add ascorbic acid the last when making the solution. 5-ethynyl-2'-deoxyuridine(EdU) is an analog of 5-methyl-2′-deoxyuridine (thymidine). So when cell proliferate, they recognize EdU as thymidine. Because of the specific chemical characteristics, EdU can react with fluorescent azide, allowing for detection of DNA replication.