Immunofluorescence protocol to detect BrdU incorporation

1- Plate cells 24 hours in advance, add BrdU (companies supply 1000X or 500X solutions) and incubate from 2 to 6 hours depending on your experiment. For rapidly growing cells a pulse of 2 hours is enough to label a significant fraction of the cells. DO NOT CHANGE MEDIA, FRESH SERUM STIMULATE S PHASE ENTRY).

2- Fix cells with for 30 min room temperature (rt) in acid ethanol (90% ethanol, 5% acetic acid, 5% H2O) (you may fix with 4% paraformaldehyde (PFH) 15 min rt if you want to co-stain your samples with another antibody).

3- Wash with PBS.

4- Add 2M HCl and incubate 20 min rt (pure HCl is 11.6M)

5- Add 0.1 M sodium borate (Na2B4O7) pH 8.5 2 min rt.

6- Wash with PBS 5 min rt

7- If you fix cells with PFH permeabilize with 0.2% Triton X100, 3%BSA in PBS for 5min rt.

8- Wash three times with PBS/BSA 10 min each.

9- Incubate with anti BrdU antibody (dilution depends on the antibody and the cell type) in PBS/BSA (Use humidification chamber).

10- Wash three times with PBS/BSA 10 min each

11- Incubate 1 hr with secondary antibody if needed (some anti-BrdU antibodies are conjugated with FITC).

12- Wash three times, 10 min each, with PBS. Add DAPI in final wash to stain DNA.

13- Mount slides