

Labelling transcription sites in mammalian cells

Method 1:

(Adapted from Koberna et al (2000) Acta Histochem 102: 15-20)

- Rinse the coverslip in KH buffer (30mM KCl, 10mM HEPES pH 7.4) briefly.
- Dip the coverslip on a piece of tissue paper to drain off excess buffer. Put the coverslip (cells faced up) on a piece of parafilm. Pipette 50µl KH buffer containing 10mM Br-UTP (Sigma).
- Put the coverslip in a tissue culture incubator for 5 min.
- Rinse the coverslip with DMEM with FCS.
- Incubate the coverslip for 20 min. in a tissue culture incubator.
- Rinse the coverslip in PBS.
- Fix the coverslip in methanol (20min, -20°C) then permeabilise in acetone (30sec, RT).
- Air dry the coverslip for about 10 min.
- Rehydrate the coverslip with PBS for 5 min.
- Immunostain the coverslip with mouse monoclonal anti-BrdU-FITC (Roche) (1:5 diluted in PBS) for 30 min.

Method 2:

(Based on [JCB 148:283](#))

- Add 1 mM 5-fluorouridine to the culture medium to initiate labelling.
- Fix the cells 10-30min later, using 1% PFA (5min, RT) and permeabilised with 0.5% TX100 (10min, RT).
- Label the incorporated FU using anti-BrU antibody (1:500, Sigma B2531), then an anti-mouse secondary antibody.