

# Worksheet IF2

## Staining of DNA-Synthesising Cells with BrdU-FITC

### Note:

This method provides a general procedure for use with the majority of Biozol reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Please note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications.

### A) Incorporation of 5'-Bromo-2'-Deoxyuridine into DNA

5'-Bromo-2'-deoxyuridine (BrdU) is incorporated into the DNA of S-phase (DNA-synthesising) cells. BrdU is simply added to the culture medium at a final concentration of 10-20 mM together with 2'-deoxycytidine (20-50 mM). Short pulses (i.e. brief presence of BrdU in culture medium) of 10 min or less can be detected. However, for routine work pulses of 1-3 h are recommended.

### B) Direct Immunofluorescence Staining

#### a) **Monolayer Cells**

1. Wash cells, grown on slides or cover slips, twice with PBS.
2. Fix cells with cold methanol for 20 minutes at -20°C (at this stage cells may be kept for 1 month at -20°C).
3. Pre-wet dry cover slips by a short incubation in PBS.
4. Denature DNA to its single-stranded form by two consecutive treatments with 4M HCl for 15 minutes each.
5. Wash 3 times with PBS
6. Incubate with the anti-BrdU-FITC-conjugate (30 minutes, room temperature humidity chamber).
7. Wash twice with PBS.
8. Mount dry samples with standard mounting medium and evaluate the results by fluorescence microscopy.

#### b) **Suspension cells**

1. Wash cells twice with PBS (250 x g, 7 minutes).
2. Resuspend cells in 3 vol PBS (0°C) and fix cells by adding 9 vol methanol (-20°C) whilst mixing the cell suspension. Incubate for 20 minutes.
3. Denature cells by adding one equal volume of 4M HCl to fixed cell suspension (20 minutes)

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- room temperature).
4. Pellet cells and resuspend the pellet in 4 M HCl, incubate for 15 minutes.
  5. Carefully remove HCl by three washes with PBS (500 x g, 7 minutes).
  6. Incubate with the anti-BrdU-FITC conjugate (30 minutes, room temperature humidity chamber)
  7. Wash twice with PBS.
  8. Mount dry samples with standard mounting medium and evaluate the results by fluorescence microscopy.

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