

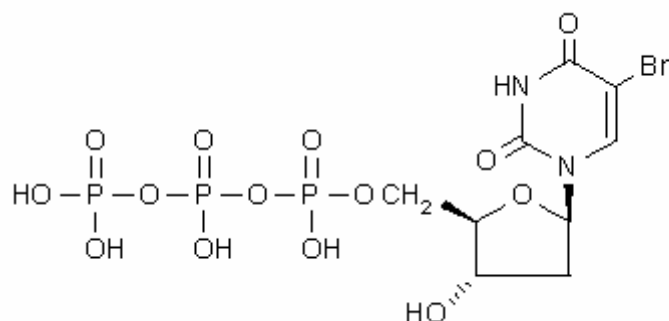
# Genaxxon BioScience

## BUdR triphosphate

5-Bromo-2'-Deoxyuridine-5'-triphosphate, Tri(ethylammonium) salt

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Product	Cat#	Package size
BUdR triphosphate (100 mM solution)	M3425.0100	100 µl
BUdR triphosphate (100 mM solution)	M3425.0500	500 µl



Picture of free acid!

### Product description

BUdR can be used for incorporation into DNA for non-radioactive DNA labelling. BUdR can be enzymatically incorporated into DNA via nick-translation, random priming, 3'-end terminal labelling or by PCR reaction.

After incorporation of BUdR, labelled DNA can be detected with BUdR antibodies.

BUdR incorporation can also be used to insert "random mutations" into DNA.

### Purity

Higher than 96% (by ion-exchange chromatography, TLC, NMR and UV). Substance dissolved in water.

### Specifications

100 mmolare solution

C<sub>27</sub>H<sub>55</sub>N<sub>5</sub>BrO<sub>14</sub>P<sub>3</sub>

MW = 846.52 g/mole (547.04 g/mole for free acid)

### Stability

BUdR is stable for more than 12 months if stored at -20°C. Repeated "freeze-thaw" cycles should be avoided.

### Storage

Store after delivery at -20°C

## Staining of BrdU-labelled DNA in proliferating Cells using mAB Bu5.1

During S-phase (DNA synthesizing phase) of cells 5'-Bromo-2'-Desoxyuridine (BrdU) is incorporated into the newly synthesized DNA.

With mAB Bu5.1 the proportion of cells in the S-phase of the cell cycle can be easily identified as mAB Bu5.1 is specific for BrdU-substituted DNA.

### Procedure

BrdU is added to the culture medium at a final concentration of 10 – 20 mM together with 2'-desoxycytidine (20 – 50 µM). For routine work pulses of 1 -2 hours are recommended.

Short pulses (e.g. brief incubation with BrdU in the cell culture medium) of 10 min. are detectable.

### Indirect Immunofluorescence Microscopy

#### Monolayer cells

1. Wash cells, grown on slides or cover slides or slips, twice with PBS.
2. Fix cells with cold 70% ethanol for 20 min. (at this stage, cells may be kept for 1 month at -20°C)
3. Denature DNA with 2.5 N HCl for 20 min.
4. Was 3x with PBS.
5. Incubate with mAB Bu 5.1 for 30 minutes at RT.
6. Was 3x with PBS.
7. Add fluorochrome-conjugated second antibody (e.g. goat anti-mouse FITC conjugate) in appropriate dilution (incubate for 30 minutes).
8. Wash 3x with PBS
9. Mount dry samples with standard mounting medium and evaluate with fluorescence microscope.

#### Cells in suspension

1. Wash and spin cells twice with PBS (250 x g for 7 minutes).
2. Resuspend cells in 3 vol. PBS (0°C) and fix cells by adding 7 vol. 96% ethanol (0°C) whilst mixing the cell suspension. Incubate for 20 minutes.
3. Denature cells by adding an equal volume of 4N HCl to fixed cell suspension (20 minutes, RT).
4. Carefully wash and spin three times with PBS to remove HCl (250 x g for 7 minutes).
5. Staining of BrdU-substituted DNA is performed as described above under monolayers steps 5 through 9. The washing steps may be reduced in order to minimise cell loss during centrifugation.

### Direct Immunofluorescence Microscopy (with FITC conjugates)

The Genaxxon FITC labelled Anti-BrdU antibodies (A1004 and A1005) are supplied in ready-to-use form.

#### Procedure:

Process cells according steps 1 – 4 (performed as above under indirect immunfluorescence).

Cells are stained by adding 25 µl of Bu 5.1 FITC conjugate. After a 30 minute incubation, cells are washed twice, embedded and are then ready for examination.

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