

# BrUTP Labeling

## Solutions:

### PB (15ml)

100 mM KOAc	(0.15 g)
30 mM KCl	(0.18 ml of 2.5 M stock)
10 mM Na <sub>2</sub> HPO <sub>4</sub>	(0.021 g)
1 mM MgCl <sub>2</sub>	(0.015 ml of 1M stock)
1 mM Na <sub>2</sub> ATP	(0.15 ml of 0.1 M stock)
1 mM DTT	(0.012 ml of 1.25 M stock)
0.2 mM PMSF	(0.03 ml of 0.1 M stock)
+ 80 U/ml RNAsin	(0.03 ml)

### Transcription Mix

<u>Stock solution</u>	<u>Amount to add</u>
100 mM ATP	10 ul
100 mM GTP, CTP	1 ul
100 mM BrUTP	1 ul
1 mM MgCL <sub>2</sub>	10 ul
PB	1000 ul

- Wash cells 2X in room temp. PB
- Put cells on ice
- Wash in PB + 0.05% TX-100 for 2 min (5 ul 10% TX-100 + 995 ul PB)
- Wash 2X in PB at 4C
- Preincubate cells at 33C for 2 min in PB
- Remove PB and add 200 ul of transcription mix (pre-warmed to 33C) for 15 min
- Wash 2X in PB at 4C
- Wash in PB + 0.2% TX-100 for 10 min at 4C (20 ul 10% TX-100 + 980 ul PB)
- Wash 2-3X in PB at 4C
- Fix as desired
- Wash 2X in PB
- Wash 2X in PBS
- Immunolabel using anti-BrdU (Boehringer) diluted 1:20 in PBS (20 ul + 380 ul)
- Wash 3X in PBS for 5 min each time
- Label with Jackson anti-mouse-TR diluted 1:250 in PBS (4 ul + 996 ul)

- Label with 1 ug/ml DAPI in dH<sub>2</sub>O for 10 min
- Mount in MOWIOL + DABCO