Labelling DNA with EdU for flow cytometry detection - fission yeast

1. Label (tk+ ENT+) strain[1] with (1-10µM) EdU - can use YES or EMM. Cells seem to only incorporate for one cycle and show checkpoint arrest (i.e. only one strand can be labelled)[2].
2. Fix cells (OD600 0.1-0.5) by spinning down and resuspending in 70% etoh. Transfer to 1.5 ml eppendorf. Cells can be stored at this stage (4°C up to a week).
3. Rehydrate by adding cells (ca. 0.2 ml, about 2 x 10^6 cells) to 5 ml PBS and spin down. Repeat wash PBS x 1 (this extra wash may not be necessary). If necessary split cells at this stage e.g. for sytox green determination of DNA content.
4. Spin down cells and pour off PBS

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5. Assemble Click it reaction components as follows:

- 1X ClickIT reaction buffer (diluted from x10 stock in kit - keep @ 4°C)
- CuSO4 (100mM)
- Fluorescent azide (kept at -20°C)
- 1X Reaction buffer additive (dilute from x10 stock in kit by diluting with deionized water)

6. Prepare appropriate volume of Click it cocktail as follows:

Table 3. Click-iT™ reaction cocktails.

Reaction components	Number of reactions						
	1	2	5	10	15	30	50
1X Click-iT™ Reaction Buffer (prepared in step 1.5)	438 µL	875 µL	2.19 mL	4.38 mL	6.57 mL	13.2 mL	21.9 mL
$CuSO_4$ (Component H)	10 µL	20 µL	50 µL	100 µL	150 µL	300 µL	500 µL
Fluorescent dye azide (prepared in step 1.3)	2.5 µL	5 µL	12.5 µL	25 µL	37.5 µL	75 µL	125 µL
Reaction Buffer Additive (prepared in step 4.6)	50 µL	100 µL	250 µL	500 µL	750 µL	1.5 mL	2.5 mL
Total volume	500 µL	1 mL	2.5 mL	5 mL	7.5 mL	15 mL	25 mL

Add 0.5 ml click it cocktail to cell pellet from step 4. Vortex. Incubate in dark at room temp for 30 mins. Go to step 7

ALTERNATIVELY

5. Make up buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM CuSO4, 10 µM Alexa Fluor 488 azide (added from 2mM stock in DMSO; A10266, Invitrogen), 10mM sodium ascorbate (added last from 0.1 M stock)

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[1] Strains that are just TK+ (ie no hENT) can be used but a much higher conc of EdU must be used (see Hua and Kearsey 2011)

[2] EdU labelling seems to cause DNA damage and activates the DNA damage checkpoint, making labelling experiments over more than one cell cycle problematic. The kinetics of S phase appear not to be altered by EdU incorporation.

6. Add 0.5 ml of this mix to cell pellet from step 4 and resuspend. Incubate in dark at room temp for 30 mins.

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7. Spin down cells, discard supernatant and resuspend in 5 ml PBS.
8. Repeat PBS wash by spinning cells down again, and resuspending in 1ml PBS
9. Finally resuspend in 1 ml PBS
10. Transfer to flow cytometry tube. Sonicate to break up doublets (use Bioruptor bath sonicator, 20s, low power. ***Do not use*** Soniprep tip soncator).
11. Sample is ready for analysis.

12. Samples can be examined by fluorescence microscopy by concentrating and mounting in 1.2% agarose (containing DAPI if required).

Manual from invitrogen:
http://probes.invitrogen.com/media/pis/mp35002.pdf

REFERENCE

Hua, H., and S.E. Kearsey. Monitoring DNA replication in fission yeast by incorporation of 5-ethynyl-2'-deoxyuridine. 2011. *Nucleic Acids Res*. 39:e60.