

Materials List for:

Chromosome Replicating Timing Combined with Fluorescent *In situ* Hybridization

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Materials

Name	Company	Catalog Number	Comments
Anti-BrdU-FITC	Roche Millipore	11202593001 MAB326F	50 µg/µl
Nick Translation Kit	Abbott Molecular (Vysis)	07J00-0001	
Spectrum Orange dUTP	Abbott Molecular (Vysis)	02N33-050	
CEP	Abbott Molecular (Vysis)	Varies	
LSI/WCP hybridization buffer	Abbott Molecular (Vysis)	06J67-011	
CEP hybridization buffer	Abbott Molecular (Vysis)	07J36-001	
Chromosome paints	MetaSystems Group	D-14NN-050-TR	
Olympus BX61 Fluorescent Microscope	Olympus	BX61TRF-1-5	
Microscope imaging software system	Applied Imaging	Cytovision 3.93.1	
Digital Camera	Olympus	UCMAD3	

IN SITU HYBRIDIZATION RECIPES

Formamide Solutions

70% Formamide/2x SSC

35 ml Formamide* (Sigma)
 10 ml 10x SSC
 5 ml d₂H₂O
 pH to 7.0 with HCl (Sigma)

*** It is important to use formamide that has been stored at -20 °C. Prolonged room temperature storage will generate formic acid and the pH will be too low.**

50% Formamide/2x SSC

25 ml formamide (Sigma)
 10 ml 10x SSC
 15 ml dH₂O
 pH to 7.0 with HCl (Sigma)

20x SSC, 4 L

702 g NaCl (Sigma)
 358 g Na Citrate (Sigma)
 dH₂O to volume

PN Buffer [0.1 M NaPO₄ 0.1% NP₄₀ (Sigma)]

Make a 0.1 M solution each of sodium phosphate (**Filter sterilize and store in 500 ml aliquots**).

0.1 M NaH₂PO₄, 1 L

13.8 g NaH₂PO₄ (Sigma):
 dH₂O to volume

0.1 M NaH₂PO₄ 1 L

14.2 g NaH_2PO_4 (Sigma)
dH₂O to volume.

PN: Adjust pH of 0.1 M Na_2HPO_4 to pH 8.0 with .1 M NaH_2PO_4 . Filter sterilize and add 1 ml of NP-40.

PNM 50 ml

1.25 g Non-fat dry milk (Sigma)
25 ml PN buffer (Recipe above)

Mix for 15-20 min with constant stirring. Spin 2 times at 400 x g for 10 min. Use supernatant, and make sure not to disturb precipitated milk proteins.