Materials List for:

Radioactive *in situ* Hybridization for Detecting Diverse Gene Expression Patterns in Tissue

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Materials

Name	Company	Catalog Number	Comments
Acetic anhydride	VWR	MK242002	
Chloroform	VWR	BDH1109	
Cresyl violet acetate	Sigma	C5042	
Cryostat	Thermo scientific	Microm HM550	
Deionized formamide	Sigma	F9037	
DTT	Promega	P1171	100mM
EDTA	Sigma	ED	
Embedding mold	VWR	15160-215	
Fisher brand Superfrost plus slide	Fisher Scientific	22-034-979	
Formaldehyde	VWR	BDH0506-4LP	
Formamide	Sigma	F7508	
GENECLEAN kit	Q-Bio gene	1001-200	
Kodak BioMax MR film	Sigma	Z350370	
Kodak NTB Emulsion	Carestream Health	8895666	
Kodak Professional developer D19	Kodak	1462593	
Kodak professional Fixer	Kodak	1971746	
β-mercapt–thanol	Calbiochem	444203	
Mineral oil	VWR	IC15169491	
NaOH	VWR	SX0600-1	
Paraformaldehyde	Sigma	76240	
Poly A	Invitrogen	POLYA.GF	
rATP	Promega	P1132	10mM
rCTP	Promega	P1142	10mM
rGTP	Promega	P1152	10mM
RNasin	Promega	N2111	40Units/μl
S^{35} UTP	PerkinElmer	NEG039C001MC	
Safety-Solve solution	Safety Solve Research Products International	111177	
Sodium Acetate	Sigma	S7899	3M
Sodium phosphate dibasic	Sigma	S3264	
Sodium phosphate monobasic	Sigma	S3139	
SP6 RNA polymerase	Promega	P1085	
Staining metal rack	Electron Microscopy Sciences	70312-54	

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T7 RNA polymerase	Promega	P2075	
Tissue-Tek OCT	Sakura	4583	
5x Transcription buffer	Promega	P1181	
Triethanolamine	VWR	IC15216391	
Tris-HCl (1 M, pH 8.0)	VWR	101449-446	
tRNA	Roche	10109509001	

Solutions:

1. Reagents for making of riboprobes: 1.5 µl DNA template (0.2-0.3 µg/µl), 2 µl of 5x optimized transcription buffer, 1 µl of 100mM DTT (comes with Polymerase from Promega, mix well at room temperature), 0.3 µl of RNasin (40 units/µl), 1.5 µl of AGC ribonucleotide mix solution, 3.5 µl of S^{35} UTP, and 1 µl RNA polymerase. Bring up to 10 µl with nuclease-free water.
2. AGC ribonucleotide mix solution: mix equal amounts of 10mM ATP, GTP and CTP together.
3. Sodium acetate solution for EtOH precipitation to remove free S^{35} UTP: 40 µl RNase and DNase free water, 5 µl of 3M Sodium Acetate, and 125 µl of 100% EtOH.
4. Hybridization buffer (10ml stock): 5 ml of 100% deionized formamide, 600 µl of 5M NaCl, 1M tris-HCl (pH = 8.0), 240 µl of 0.5M EDTA (pH =8.0), 100 µl of 100x Denhart's solution, 100 µl of 1M DTT, 250 µl of 20mg/ml tRNA, 125 µl of 20 mg/ml poly A, and 1 g of Sodium Dextran Sulfate. Add DEPC-treated water to bring the total volume to 10 ml. Shake vigorously and then incubate at 55 °C until all sodium dextran sulfate is dissolved. Store the hybridization buffer in -20 °C, which is good for ~6 months.
5. 4% buffered paraformaldehyde solution: Add 40 g of paraformaldehyde in 760 ml distilled water in a flask designated for paraformaldehyde, heat to 50 °C on a hot plate while stirring. Add 320 µl of 10N NaOH to help dissolve the paraformaldehyde. After dissolving (~10 min), add 100 ml of 10x PBS, and bring the volume up with distilled water until 1 liter. Stir and heat the solution until paraformaldehyde is dissolved. The pH should be 7.4.
6. Acetylation buffer: 13.6 ml of triethanolamine plus 2.52 ml of acetic anhydride in 1 liter of distilled water.
7. 20x SSPE solution: 3M NaCl, 200 mM NaH_2PO_4-H_2O, and 200 mM EDTA in distilled water. Adjust solution to pH 7.4 with 10N NaOH.
8. 10x PBS buffer: 80g of NaCl, 2.0g of KCl, 14.4g of Na_2HPO_4, and 2.4g of KH_2PO_4 in distilled water. Adjust solution to pH 7.0 and bring total volume to 1 liter.
9. Second wash solution: 0.1% β-mercapt–thanol and 50% formamide in 2x SSPE
10. Cresyl violet acetate solution: 3% cresyl violet acetate in tap water (distilled water prevents good staining), dissolved overnight with stirring in a flask at room temperature. Filter with vacuum suction through a 1mm Whatman filter paper and Buchner funnel.

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