**Materials List for:**

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

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URL: http://www.jove.com/video/3764/

## 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-mercaptoethanol | 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 | |
| T7 RNA polymerase | Promega | P2075 | |

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|---|---|---|---|
| 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-mercaptoethanol 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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