

Materials List for:

Enhanced Northern Blot Detection of Small RNA Species in *Drosophila Melanogaster*

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Materials

Name	Company	Catalog Number	Comments
FINAL COMPOSITION/NAME	COMPANY	CATALOGUE (Stock)	COMMENTS
GENERAL REQUIREMENT			
Centrifuge, 5418	Eppendorf	5418 000.017	
Decontaminant, RNase Away	Sigma-Aldrich	83931	Apply on glass/plasticware, wipe and rinse
RNase inhibitor, DEPC	Sigma-Aldrich	D5758	Hazardous //// 1609-47-8
SAMPLE PREPARATION			
Stereo Microscope, M60	Leica		
1X PBS Buffer			
137 mM NaCl	Sigma-Aldrich	S3014	7647-14-5
2.7 mM KCl	Sigma-Aldrich	P9541	7447-40-7
10 mM Na ₂ HPO ₄	Sigma-Aldrich	S3264	7558-79-4
1.8 mM KH ₂ PO ₄	Sigma-Aldrich	P9791	7778-77-0
RNA EXTRACTION/ANALYSIS			
UV-Vis Spectrophotometer	Thermo Scientific	Nanodrop 2000	
Gel Imager, ChemiDoc XRS+	Biorad	170-8265	
Horizontal Electrophoresys, Mini-Sub Cell GT	BioRad	170-4487EDU	
RNA extraction reagent, TRIzol Reagent	Life Technologies	15596026	Hazardous
PCA (25:24:1), 1:1 for extraction	Sigma-Aldrich	77617	136112-00-0
Chlorophorm, 1:1 for extraction	Sigma-Aldrich	C2432	67-66-3
EtOH, 2.5 vol. for precipitation, 70% for washing	Sigma-Aldrich	59844	64-17-5
Gene Ruler 1Kb DNA Ladder	Thermo Scientific	SM0311	
Stop Mix			
300 mM NaOAc, pH 5.5	Sigma-Aldrich	S2889	127-09-3
1x RSB Solution			
2% sodium dodecyl sulfate (SDS)	Sigma-Aldrich	71736	Never cool down SDS to avoid precipitation //// 151-21-3
2 mg/ml Proteinase K	Roche Applied Science	0-3115828001	EC 3.4.21.64 9
10X Reticulocyte Standard Buffer (RSB)			
100 mM Tris-Cl, pH 7.4	Sigma-Aldrich	T5941	1185-53-1
100 mM NaCl	Sigma-Aldrich	S3014	7647-14-5

30 mM MgCl ₂	Sigma-Aldrich	M8266	7786-30-3
Denaturing Agarose Gel			
1.2% Agarose	Sigma-Aldrich	A9539	Let the gel solidify for at least 1 hr //// 9012-36-2
10X MOPS Buffer			
0.2 M MOPS sodium salt, pH 7	Sigma-Aldrich	M9381	Do not confuse MOPS sodium salt with MOPS //// 71119-22-7
Agarose Loading Dye			
1x MOPS Buffer			
3% formaldehyde	Sigma-Aldrich	F8775	Hazardous //// 50-00-0
50% formamide	Sigma-Aldrich	47670	Hazardous //// 75-12-7
Ethidium bromide (EtBr) 30 µg/ml	Sigma-Aldrich	E1510	Hazardous //// 1239-45-8
Bromophenol blue	Sigma-Aldrich	B0126	115-39-9
SMALL RNA FRACTIONATION			
Flat gel loading tips	Life Technologies	LC1002	
Savant SpeedVac Concentrator	Thermo Scientific	DNA120	
Vertical Electrophoresis, Mini-PROTEAN Tetra Cell	Biorad	165-8000	
Denaturing Acrylamide Gel			
10% acrylamide/bis-acrylamide (19:1)	Sigma-Aldrich	A3449	Hazardous. Let the gel polymerize for 45 min before use
1x TBE Buffer (or 1x MOPS Buffer)			
7 M urea	Sigma-Aldrich	U6504	57-13-6
0.1% ammonium persulfate	Sigma-Aldrich	215589	7727-54-0
0.1% TEMED	Sigma-Aldrich	T9281	110-18-9
Gene Ruler Ultra Low Range DNA Ladder (10 bp-step)	Thermo Scientific	SM1211	Label 0.1 µg, as described in the "probe labeling" reaction
Acrylamide Blue Dye			
50% formamide	Sigma-Aldrich	47670	Hazardous //// 75-12-7
Bromophenol blue	Sigma-Aldrich	B0126	115-39-9
Running Buffer (RB)			
1x MOPS Buffer			
Alternative RB: 1x TBE Buffer			
1x TBE Buffer			
5X TBE			
445 mM Tris-base	Sigma-Aldrich	T1503	77-86-1
445 mM boric acid	Sigma-Aldrich	B7901	10043-35-3
20 mM EDTA	Sigma-Aldrich	EDS	60-00-4
Gel Staining Solution			
Ethidium bromide 0.5 µg/ml	Sigma-Aldrich	E1510	Hazardous //// 1239-45-8
1x RB			Stain for 15 min with EtBr, destain for 15 min w/o EtBr
BLOTTING			
3MM Whatman paper	Sigma-Aldrich	Z270849	
Neutral Nylon Membrane, Hybond NX	GE Healthcare Life Science	RPN203T	Photosensitive
CROSSLINKING			
Crosslinking Solution (XLS)			

1% 1-methylimidazole (v/v)	Sigma-Aldrich	336092	Always prepare a fresh aliquot of XLS //// 616-47-7
12.5 mM HCl	Sigma-Aldrich	H1758	Hazardous //// 7647-01-0
3.1% EDC (w/v)	Sigma-Aldrich	3450	25952-53-8
(PRE)-HYBRIDIZATION			
Liquid Scintillation Counter	Beckmann	LS 6500	
Sheared Salmon Sperm, 0.1 mg/ml	Life Technologies	AM9680	
rasi4 anti-sense probe			5' CGGUGUUCGACAGUCCUCGGG -3'
5s-rRNA anti-sense probe			5'- CAACACGCGGTGTTCCCAAGCCG-3'
Dmel-miR279 anti-sense probe			5'- TTAATGAGTGTGGATCTAGTCA-3'
Dmel-miR286 anti-sense probe			5'- AGCACGAGTGTTCGGTCTAGTCA-3'
Purification Kit, Illustra MicroSpin G-25 Columns	GE Healthcare Life Science	27-5325-01	
(Pre)-Hybridization Solution (HS)			
0.5% SDS	Sigma-Aldrich	71736	151-21-3
5x Denhardt's Solution			
20X SSPE			
3.6 M NaCl	Sigma-Aldrich	S3014	7647-14-5
0.2 M sodium phosphate	Sigma-Aldrich	342483	7601-54-9
20 mM EDTA (pH 8.0)	Sigma-Aldrich	EDS	60-00-4
50x Denhardt's Solution			
1% Ficoll 400 (w/v)	Sigma-Aldrich	F2637	26873-85-8
1% Polyvinylpyrrolidone	Sigma-Aldrich	PVP40	9003-39-8
1% BSA	Sigma-Aldrich	A7906	9048-46-8
1X TEN Buffer			
10 mM Tris-Cl (pH 8.0)	Sigma-Aldrich	T1503	77-86-1
100 mM NaCl	Sigma-Aldrich	S3014	7647-14-5
1 mM EDTA (pH 8.0)	Sigma-Aldrich	EDS	60-00-4
Probe Labelling			
0.4 µM oligonucleotide			
1.5 µl Ci/µl [γ -32P] ATP	Perkin Elmer	NEG002A	Hazardous //// 51963-61-2
1x T4 Polynucleotide Kinase Buffer	Biolabs	B0201	
T4 Polynucleotide Kinase 0.5 units/µl	Biolabs	M0201	EC 2.7.1.78
WASHING			
Geiger Mueller Detectors	Canberra Industries	MCB2/CPS – EM77021	
Washing Buffer (WB), 2x SSPE			
Stringent Washing Buffers			
0.2x SSPE			
2x SSPE, 0.5% SDS			
0.2x SSPE, 0.5% SDS			
SIGNAL DETECTION			
PharosFX Plus System	Biorad	170-9460	

Image J	Freely available		
Quantity One	Biorad		
X-ray films , BioMax XAR	Sigma-Aldrich	F5888	Photosensitive
Developer for X-ray films	Sigma-Aldrich	P7042	
Fixer for X-ray films	Sigma-Aldrich	P7167	
STRIPPING			
Stripping Solution			
10 mM Tris-HCl pH 8.8	Sigma-Aldrich	T1503	77-86-1
5 mM EDTA	Sigma-Aldrich	EDS	60-00-4
0.1% SDS	Sigma-Aldrich	71736	Add SDS after boiling to avoid foaming //// 151-21-3