Materials List for:

Method for the Isolation and Identification of mRNAs, microRNAs and Protein Components of Ribonucleoprotein Complexes from Cell Extracts using RIP-Chip

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Materials

Name	Company	Catalog Number	Comments
1 M dithiothreitol	Fisher	BP172-5	DTT
DNase 1	Ambion	2235	RNase free
Ethylendiamine Tetraccetic Acid	Fisher	BP118-500	EDTA
Glycogen	Ambion	9516	
1 HEPES	Sigma	H3375-100G	pH 7.0
Igepal Nonidet P-40	USB	78641	NP40
1 M KCl	Fisher	BP366-500	
1 M $MgCl_2$	Fisher	BP214-500	
NT2 Buffer	*See Below		
Polysome Lysis Buffer	*See Below		
Protease inhibitor cocktail tablets	Roche	11873580001	
Protein A Sepharose Beads	Sigma	P3391	
Proteinase K	Fisher	BP1700-100	
RNase Out RNase inhibitor	Invitrogen	10777-019	40 U/µl
1 M NaCl	Fisher	BP358-212	
Sodium dodecyl sulfate	Fisher	BP166-500	SDS
1 M Tris-HCl	Fisher	BP153-500	pH 7.4
Trizol	Invitrogen	15596-026	
Vanadyl Ribonucleoside Complexes	New England Labs	S1402S	VRC
Reagent Workup			
Prepare reagents in RNase/ DNase-free, DEPC-treated glassware			
Polysome lysis Buffer			
100 mM KCl			
5 mM $MgCl_2$			
10 mM HEPES (pH 7.0)			
0.5% NP40			
1 mM DTT			
100 units/ml RNase Out			
400 µM VRC			

Protease inhibitor cocktail tablet			
5 ml of Polysome lysis buffer			
Add 50 µl of 1 M HEPES (pH 7.0)			
500 µl of 1 M KCL			
25 µl of 1 M $MgCl_2$			
25 µl of NP40			
4.7 ml RNase-DNase-free H_2O			
50 µl of 1 M DTT			
12.5 µl of 100 U/ml RNase Out			
200 µl Protease inhibitor cocktail (dissolved according to manufacturer)			
10 µl 200 mM VRC (at time of use)			
NT2 Buffer			
50 mM Tris-HCl (pH 7.4)			
150 mM NaCl			
1 mM $MgCl_2$			
0.05% NP40			
1 L of NT2 Buffer			
50 ml Tris (pH 7.4)			
30 ml 5 M NaCl			
1 ml 1 M $MgCl_2$			
500 µl NP40			
820 ml RNase-DNase-free H_2O			