

Materials List for:

Small-scale Nuclear Extracts for Functional Assays of Gene-expression Machineries

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Materials

Name	Company	Catalog Number	Comments
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid	Sigma	H3375-500G
MgCl ₂ ·6H ₂ O	Magnesium Chloride Hexahydrate	Sigma	M2670-500G
KCl	Potassium Chloride	Fisher	P335-212
PMSF	Phenylmethanesulfonyl fluoride	Sigma	P7626-100G
DTT	Dithiothreitol	American Bioanalytical	AB00490-5G
EDTA	Ethylenediaminetetraacetate, disodium, dihydrate	American Bioanalytical	AB00500-01000
Glycerol		MP Biomedicals	800689
DMEM	Dulbecco's Minimal Essential Medium	Invitrogen	11995-073
FBS	Fetal Bovine Serum	Gibco	16140
Penicillin/Streptomycin		Gibco	15070
PBS	Phosphate Buffered Saline	Cellgro	21-040-CV
Trypan Blue Stain 0.4%		Gibco	15250
Cell Lifters		Corning	29442-200
150mm Plates		VWR	353025
Extended Micropipette Tip		Denville	P1126
Dounce homogenizer, 1ml, with tight pestle		Wheaton	357538
Slidealizers, MWCO 10kDa		ThermoScientific	69572
Mini-Centricons, MWCO 3kDa		Millipore (Amicon)	UFC500396

Table 1. Specific Reagents and Equipment.

Hypotonic	10 mM HEPES, pH 7.9	1M HEPES, pH 7.9	5 ml
	1.5 mM MgCl ₂	1M MgCl ₂	750 µl
	10 mM KCl	3M KCl	1.67 ml
	0.2 mM PMSF	200 mM PMSF	500 µl
	0.5 mM DTT	2M DTT	125 µl
Bring up to 500 ml with water			
Low Salt	20 mM HEPES, pH 7.9	1M HEPES, pH 7.9	2 ml
	1.5 mM MgCl ₂	1M MgCl ₂	150 µl
	20 mM KCl	3M KCl	667 µl
	0.2 mM EDTA	0.5M EDTA	40 µl
	25% Glycerol	Glycerol	25 ml
	0.2 mM PMSF	200 mM PMSF	100 µl

	0.5 mM DTT	2M DTT	25 μ l
Bring up to 100 ml with water			
High Salt	20 mM HEPES, pH 7.9	1M HEPES, pH 7.9	2 ml
	1.5 mM $MgCl_2$	1M $MgCl_2$	150 μ l
	1.4M KCl	3M KCl	48 ml
	0.2 mM EDTA	0.5M EDTA	40 μ l
	25% Glycerol	Glycerol	25 ml
	0.2 mM PMSF	200 mM PMSF	100 μ l
	0.5 mM DTT	2M DTT	25 μ l
Bring up to 100 ml with water			
Dialysis	20 mM HEPES, pH 7.9	1M HEPES, pH 7.9	10 ml
	100 mM KCl	3M KCl	16.625 ml
	0.2 mM EDTA	0.5M EDTA	200 μ l
	20% Glycerol	Glycerol	100 ml
	0.2 mM PMSF	200 mM PMSF	500 μ l
	0.5 mM DTT	2M DTT	125 μ l
Bring up to 500 ml with water			
Table 2. Solutions for Nuclear Extract Preparation.			