

Materials List for:

An Oligonucleotide-based Tandem RNA Isolation Procedure to Recover Eukaryotic mRNA-Protein Complexes

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Materials

Name	Company	Catalog Number	Comments
MaxQ 5000 Large Incubated and Refrigerated Orbital Shaker	Thermo Fisher Scientific	SHKE5000-8CE	Floor shaker to grow yeast cells
BBD 6220	Thermo Fisher Scientific		CO ₂ incubator to grow human cells
Sonicator Soniprep150	MSE	MSS150.CX4.5	Sonicator/cell disruptor. Used to shear DNA in human cell lysates
Stratalinker 1800	Stratagene		Stratalinker to expose cells to UV light at 254 nm
Tissue Lyser	Qiagen	RETSCH MM200	Device to mechanically disrupt yeast cells
Refrigerated Centrifuge	Eppendorf	5810R	Centrifuge to spin down cells, lysates
Shaking incubator, Thriller	Peqlab		Thermoshaker for 1.5 mL tubes
Glass beads 0.5mm	Stratech Scientific Limited	11079105	Lysis of yeast cells
Nylon Filter, 0.41 µm	Millipore	NY4104700	Collection of yeast cells
Millex-HA Filter, 0.45 µm	EMD Millipore	SLHA02510	Filter to clear nematode lysate
Eppendorf LoBind microcentrifuge tubes Protein 1.5 mL	Sigma-Aldrich	22431081	Minimise protein loss
2 mL microfuge tube	Ambion	AM12425	Yeast extract preparation and other applications
5 mL tube	Thermo Fisher Scientific	129A	Collection of HEK293 cell lysate
15 mL tube	Sarstedt	62.547.254	Collection <i>C. elegans</i>
50 mL plastic tube	Sarstedt	62.554.502	Collection yeast cells
DMEM, high glucose, pyruvate	Thermo Fisher Scientific	41966	Media for culturing HEK293 cells
Penicillin-Streptomycin	Sigma-Aldrich	P4333	Used to supplement cell culture media to control bacterial contamination
Fetal Bovine Serum (FBS)	Sigma-Aldrich	F7524	Used to supplement cell culture media
Lipofectamine 2000	Thermo Fisher Scientific	11668027	Transfection reagent
RQ1 RNase-Free DNase	Promega	M6101	DNase I to degrade DNA from cell lysate
RNasin Plus RNase Inhibitor	Promega	N2611	RNase inhibitor to avoid RNA degradation
cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail	Roche	11836170001	Protease inhibitor cocktail to avoid protein degradation
Dynabeads Oligo (dT)25	Thermo Fisher Scientific	61011	Magnetic beads required for the first step of mRNA-protein complex purification

Dynabeads M-280 Streptavidin	Thermo Fisher Scientific	11205D	Magnetic beads required for the second step of mRNA-protein complex purification
<i>E. coli</i> tRNA	Sigma-Aldrich	10109550001	transfer RNA from <i>E. coli</i> used for blocking streptavidin beads and avoid unsepecific interactions
Quick Start Bradford 1x Dye Reagent	BioRad	5000205	To determine protein concentration within lysates, using BSA as reference
Bovine Serum Albumin	Sigma	A7906-100G	Used to perform the standard curve that will be used as reference for protein concentration determination
mouse anti-Act1	MP Biomedicals	869100	Antibody for detection of yeast actin in Western blot (1:2,500)
mouse anti-Act1	Sigma	A1978	Antibody for detection of human actin in Western blot (1:2,000)
mouse anti-HuR	Santa Cruz	sc-5261	Antibody for detection of HuR in Western blot (1:500)
mouse anti-CYC-1	Invitrogen	456100	Antibody for detection of Cyc-1 in Western blot (1:1,000)
peroxidase anti-peroxidase soluble complex	Sigma	P1291	Detection of Pfk2:TAP in Western blot (1:5,000)
HRP-conjugated sheep anti-mouse IgG	Amersham	NXA931	HRP-coupled secondary antibody