

Materials List for:

Desthiobiotin-Streptavidin-Affinity Mediated Purification of RNA-Interacting Proteins in Mesothelioma Cells

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Materials

Name	Company	Catalog Number	Comments
NE-PER Nuclear and cytoplasmic extraction kit	Thermo Fisher Scientific	78833	
Pierce Protease Inhibitor Tablets, EDTA-free	Thermo Fisher Scientific	A32965	
Pierce BCA Protein Assay Kit	Thermo Fisher Scientific	23225	
Pierce Magnetic RNA-protein Pull-Down Kit	Thermo Fisher Scientific	20164	Includes magnetic beads and therefore the kit need to be stored at 4 °C
Pierce RNA 3' End Desthiobiotinylation Kit	Thermo Fisher Scientific	20163	The kit is a part of the "Pierce Magnetic RNA-protein Pull-Down Kit", and needs to be stored at -20 °C unlike the pull-down kit (4 °C).
DynaMag-2, magnetic stand	Thermo Fisher Scientific	12321D	
Anti - HuR (MOUSE) monoclonal antibody	Thermo Fisher Scientific	-	The antibody is included in the Pierce Magnetic RNA-protein Pull-Down Kit - 1:1000 in 5% BSA 1x TTBS - rabbit anti-mouse secondary antibody 1:10,000 in 5% BSA 1x TTBS
Anti - α - tubulin (MOUSE) monoclonal antibody	Santa Cruz	8035	1:1000 in 5% BSA 1x TTBS - rabbit anti-mouse secondary antibody 1:10,000 in 5% BSA 1x TTBS
Anti - PARP (RABBIT) Polyclonal antibody	Cell Signaling	9542	1:1000 in 5% BSA 1x TTBS - goat anti-rabbit secondary antibody 1:10,000 in 5% BSA 1x TTBS
Anti - Mesothelin (MOUSE) Monoclonal Antibody	Rockland	200-301-A87	
Secondary goat anti-rabbit antibody	Cell Signaling	7074	
Secondary rabbit anti-mouse antibody	Sigma-Aldrich	A-9044	
RPMI - 1640 medium	Sigma-Aldrich	R8758	
FBS - Filtrated Bovine Serum	Pan Biotech	P40-37500	
Penicillin - streptomycin (100x)	Sigma-Aldrich	P4333	
L-Glutamine solution (200 mM)	Sigma-Aldrich	G7513	
0.25% Trypsin-EDTA (1x)	Gibco by Life technologies	25200-056	
Mini-PROTEAN 3 Cell	Bio-Rad	1653301	
Mini Protean System Glass Plates	Bio-Rad	1653308	
Mini-PROTEAN Spacer Plates with 1.5 mm integrated spacer	Bio-Rad	1653312	

Mini-PROTEAN Comb, 10-well, 1.5 mm, 66 µL	Bio-Rad	1653365	
Mini-PROTEAN Tetra Cell Casting Modules	Bio-Rad	1658050	
RNaseZap RNase Decontamination Solution	Thermo Fisher Scientific	AM9780	
Rotiphorese gel 30 - aqueous 30% acrylamide and bisacrylamide stock solution at a ration of 37.5:1	Roth	3029	
20% SDS	PanReac AppliChem	A3942	
PVDF transfer membrane	Perkin Elmer	NEF1002	Need to be activated by incubating in pure methanol for 1 min followed by washing in water for 2 min
Trans-Blot SD semi-dry transfer cell	Bio-Rad	1703940	
Clarity Western ECL Substrate	Bio-Rad	1705061	
FusionFX Digital Imager	Vilber	-	
RNA oligo synthesis	Mycrosynth AG, Switzerland	-	the synthesis scale - 0.04 µmol - HPLC purified
Bovine serum albumin	Sigma-Aldrich	A7030	
Hydrochloric Acid (HCl) 6 M	PanReac AppliChem	182883.1211	
Ultra Pure 1 M Tris, pH 7.5	Thermo Fisher Scientific	15567027	
Glycerol	Thermo Fisher Scientific	17904	50% sterile aliquots to be stored at -20°C
Pierce Streptavidin magnetic beads	Thermo Fisher Scientific	88817	Please note that these magnetic beads have an average diameter of 1 µm (range from 0.5 - 1.5 µm). Furthermore, Dynabeads-M280 Streptavidin, which are beads of homogenous size 2.8 µm, are also used in RNA-pulldown but be aware that this may affect purification process.
TEMED	Sigma-Aldrich	T9281	
Ammonium persulfate	Sigma-Aldrich	A3678	
Coomassie G-250	Applchem	A3480	
Aluminiumsulfate-(14-18)-hydrate	Sigma-Aldrich	368458	
ortho-phosphoric acid	Applchem	A0637	