Materials List for:

Metabolic Labeling of Newly Transcribed RNA for High Resolution Gene Expression Profiling of RNA Synthesis, Processing and Decay in Cell Culture

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Materials

Name	Company	Catalog Number	Comments
4-thiouridine	Carbosynth	T4509	Prepare 50 mM stock in sterile H_2O, store at -20 °C in aliquots of 50-500 µl, discard unused reagent, do not refreeze.
Trizol	Invitrogen	15596026 (100 ml), 15596018 (200 ml)	**WARNING - CORROSIVE and HAZARDOUS TO HEALTH!** Ensure immediate access to Phenol antidote (PEG-Methanol); Store at 4 °C.
Chloroform	Sigma	372978	**WARNING - HAZARDOUS TO HEALTH**
Isopropanol	Sigma	650447	
Sodium citrate, nuclease-free	Sigma	C8532	Prepare 1.6 M stock solution using nuclease-free water.
5M nuclease-free NaCl	Sigma	71386	Stock solution
Nuclease-free H_2O	Sigma	W4502	Make 1 ml aliquots in nuclease-free tubes.
RNA precipitation buffer			1.2 M NaCl, 0.8 M sodium citrate in nuclease free water. Prepare in advance under strictly nuclease-free conditions. Store at room temperature in 50 ml falcon tubes.
Ethanol	Sigma	459844	Use with nuclease-free water to prepare 80% ethanol, store at -20 °C.
1 M nuclease-free Tris Cl, pH 7.5	Lonza	51237	Stock solution
500 mM nuclease-free EDTA, pH 8.0	Invitrogen	15575-020	Stock solution
10x Biotinylation Buffer (BB)			100 mM Tris pH 7.4, 10 mM EDTA in nuclease-free water, make aliquots of 1 ml.
Dimethylformamide (DMF)	Sigma	D4551	
EZ-Link biotin-HPDP	Pierce	21341	Prepare 1 mg/ml stock solution by dissolving 50 mg biotin-HPDP in 50 ml DMF. Gentle warming enhances solubilisation. Store at 4 °C in aliquots of 1 ml.
Phase Lock Gel Heavy tubes 2.0 ml	Eppendorf	0032 005.152	Optional for the chloroform extraction step.

Zeta membrane	BIORAD	162-0153	
10x Dot blot binding buffer			100 mM NaOH, 10 mM EDTA
Biotin-oligo			5'-biotin, 25 nucleotides, any sequence
Sodium dodecyl sulphate	Fisher	BPE9738	For 100 ml 20% stock solution, add 20 g SDS to 80 ml PBS pH 7-8 and adjust volume to 100 ml. Keep all high-percentage SDS solutions above 20 °C. Warm the solutions slightly should SDS precipitate.
EZ-Link Iodoacetyl-LC-Biotin	Pierce	21333	Prepare 1 mg/ml stock solution by dissolving 50 mg iodoacetyl-biotin in 50 ml DMF. Gentle warming enhances solubilisation. Store at 4 °C in aliquots of 1 ml. Generates irreversible, thiol-specific biotinylation.
Phosphate buffer saline	Gibco	10010-015	
Dot blot blocking buffer			Mix 20 ml 20% SDS with 20 ml 1 x PBS pH 7-8 and add EDTA to the final concentration of 1 mM.
Streptavidin-horseradish peroxidase	Vector Laboratories	SA5004	Store at -20 °C. Mix 10 ml 20% SDS with 10 ml 1 x PBS. Add 20 µl Streptavidin-HRP before use.
ECL reagent	GE Healthcare	RNP2109	Use following the manufacturer's instructions.
Super RX, X-RA Film, 18x24 cm	Fujifilm	47410 19236	
µMacs Streptavidin Kit	Miltenyi	130-074-101	Store the beads at 4 °C.
Tween 20	Sigma	P1379	
Washing buffer			100 mM Tris pH 7.4, 10 mM EDTA, 1 M NaCl, 0.1% Tween 20 in nuclease-free H_2O.
Dithiothreitol (DTT)	Sigma	43817	Prepare as 100 mM DTT in nuclease-free H_2O, always prepare fresh before use.
RNeasy MinElute Kit	Qiagen	74204	Store columns at 4 °C, remaining components of the kit at room temperature.
1.5 ml screw-top polypropylene tubes	Sarstedt	72.692.005	Compatible with Dimethylformamide
2.0 ml screw-top polypropylene tubes	Sarstedt	72.694.005	Compatible with Dimethylformamide
15 ml tubes	BD Falcon	352096	Compatible with Dimethylformamide
50 ml tubes	BD Falcon	352070	Compatible with Dimethylformamide
All solutions/reagents should be stored at room temperature unless otherwise specified.			
Equipment			
UV/VIS spectrophotometer	Thermo Scientific	NanoDrop 1000	Or equivalent. Use low volume (1-2 µl) for measurements of low RNA concentrations to avoid excessive sample loss.
Polypropylene 15 ml centrifuge tubes	VWR International	525-0153	In contrast to standard 15 ml tubes, these tolerate up to 15,000 × g
High-speed centrifuge	Beckman Coulter	Avanti J-25	Or equivalent equipment capable of reaching 13,000×g

High-speed rotor	Beckman Coulter	JLA-16250	Or equivalent equipment capable of reaching 13,000×g
Adaptors for 15 ml tubes	Laborgeräte Beranek	356964	Or equivalent equipment capable of reaching 13,000×g
Refrigerated table-top centrifuge	Eppendorf	5430 R	Or equivalent.
Thermomixer	Eppendorf	Thermomixer compact	Or equivalent.
Magnetic stand	Miltenyi Biotec	130-042-109	One stand holds 8 µMacs columns.
Waterbath	Grant	SUB Aqua 5	Or equivalent.
Ultra-fine scale	A&D	GR-202	Or equivalent.
E-Gel iBase Power System	Invitrogen	G6400UK	For RNA gels; or equivalent.
E-Gel EX 1% agarose precast gels	Invitrogen	G4020-01	For RNA gels; or equivalent.