

Materials List for:

Extremely Rapid and Specific Metabolic Labelling of RNA In Vivo with 4-Thiouracil (Ers4tU)

J. David Barrass¹, Jean D. Beggs¹

¹Wellcome Centre for Cell Biology, School of Biological Sciences, University of Edinburgh

Correspondence to: J. David Barrass at David.Barrass@ed.ac.uk

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Materials

Name	Company	Catalog Number	Comments
β-mercaptoethanol (βME)	Sigma-Aldrich	M3148	CAUTION toxic. Stock solutions are approximately 14 M, make at 1/20 dilution for use
Chloroform	Sigma-Aldrich	25668	CAUTION toxic
Diethyl pyrocarbonate (DEPC)	Sigma-Aldrich	D5758	add 1/1000 volume to a solution, leave at room temperature for 24 h, then autoclave
DMF (N,N-dimethylformamide)	Sigma-Aldrich	227056	CAUTION toxic
EDTA	Sigma-Aldrich	3609	Make 0.5 M and pH to 8.0 with sodium hydroxide
Ethanol	Sigma-Aldrich	29221	
EZ-link HPDP Biotin	Thermo scientific	21341	Store protected from light. Dissolve all the vial contents in 22.7 mL DMF (to make a 4 mM stock solution). Store away from water, in the dark & at -20 °C. Check the solution before using, as some batches of HPDP precipitate in storage; heat at 42 °C to resuspend.
Glucose	Fisher Scientific	G/0500/60	
Glycogen [20 mg/mL]	Sigma-Aldrich	10901393001	Store at -20 °C
Immobilised TCEP Disulfide Reducing Gel	Thermo Scientific	77712	Optional
LiCl	Sigma-Aldrich	793620	10 M solution. CAUTION: this gets very hot as it dissolves and can even boil at greater than 100 °C, add the LiCl crystals to the water slowly.
Magnesium chloride (MgCl ₂)	Sigma-Aldrich	63033	1 M solution. CAUTION: this gets very hot as it dissolves and can even boil at greater than 100 °C, add the MgCl ₂ crystals to the water slowly.
Methanol	Fisher Scientific	M/4000/PC17	CAUTION Toxic and flammable
NaH ₂ PO ₄	Sigma-Aldrich	S3139	Make 1 M solutions of each and mix in equal amount to obtain a solution of the appropriate pH
Na ₂ HPO ₄	Sigma-Aldrich	S3264	
NaCl	Sigma-Aldrich	S9888-M	5 M solution

Phenol, low pH.	Sigma-Aldrich	P4682	Store in the dark at 4 °C. CAUTION toxic
Phenol Chloroform 5:1 (125:24:1) low pH.	Sigma-Aldrich	P1944	Store in the dark at 4 °C. CAUTION toxic
Pierce Spin Columns	Thermo Scientific	69702	Optional
SCSM single drop-out –ura	Formedium	DSCS101	
Sodium Acetate	Sigma-Aldrich	32318-M	Make a 3 M solution and pH to 5.3 with acetic acid
Sodium hydroxide	Sigma-Aldrich	795429	CAUTION corrosive
SDS (Sodium dodecyl sulfate)	Sigma-Aldrich	436143	CAUTION irritant, do not inhale
Streptavidin Magnetic beads	NEB	1420S	Store at 4°C
SUPERase-In, RNase inhibitor	Life technologies	AM2696	Store at -20°C
Thiolated <i>Schizosaccharomyces pombe</i> for spike			See section 1.7 of the protocol
4-thiouracil (4tU)	ACROS ORGANICS	359930010	Store in the dark. Make 100 mM Stock in 1M NaOH, store solutions at -20°C.
Tris base	Sigma-Aldrich	93362	1 M solutions at various pH
tRNA	Sigma-Aldrich	10109541001	5mg/ml, store at -20°C
Uridine	Sigma-Aldrich	U3750	Make 1 M solution in H ₂ O. Split into 2 mL aliquots and store at -20 C.
Yeast nitrogen base without amino acids with amonium sulphate	Formedium	CYN0410	
Zeba Columns 0.5ml	Thermo Scientific	89882	Store at 4 °C
Zirconia beads	Thistle Scientific	110791052	
Equipment and Consumables			
Beadbeater	Biospec	112011EUR	Other homogenisers can be used; the correct conditions for each homogeniser and strain must be established.
Bioanalyser (Agilent) or similar to assess RNA quality. If this is not important a spectrophotometer is useful to quantify the RNA.			
Centrifuge: capable of spinning cultures at 4 °C and at least 3000 g. Pre-chill if possible.			
Centrifuge: capable of spinning up to 2 mL tubes at variable speeds upto 13,000 g and down to 1000 g			
Magnetic rack for separating the beads from the sample. The one used in the paper is 3D printed, available from Thingiverse (thing:3562952). Commercially available racks exist			
PCR machine with a heated lid that will allow incubation in the dark.			
Rotating wheel to rotate 1.5 mL tubes end over end			
Shaking heating block (such as Eppendorf Thermomixer) is recomended			

Tubes, centrifuge, Low retention, RNase free 0.5mL	Eppendorf	H179467N	
Tubes, centrifuge, Low retention, RNase free 1.5mL	Ambion	AM12350	
Tubes, centrifuge, 50 mL	Sarstedt	62.547.004	Other centrifuge tubes are not gas proof allowing CO ₂ to dissolve in the methanol, this comes out of solution vigorously on adding warm culture, leading to sample loss
Tubes, centrifuge, 15 mL	Sarstedt	62.554.001	
Tubes, 2 mL, screw cap	Greiner	723361	
Tubes 0.2 mL strip of 8 with integral lids	Brand	781332	