

Materials List for:

Real-time Analysis of Transcription Factor Binding, Transcription, Translation, and Turnover to Display Global Events During Cellular Activation

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Materials

Name	Company	Catalog Number	Comments
4sU-labeling			
4-thiouridine (100 mg)	Carbosynth	13957-31-8	Prepare 50 mM stock in sterile H ₂ O/PBS; store at –20°C in aliquots of 50-500 µl; do not refreeze.
1.5 ml safe-lock tubes	Eppendorf	30121589	Optional
1.5 ml screw-top polypropylene tubes	Sarstedt	72692005	Compatible with Dimethylformamide
15 ml tubes	BD Falcon	352096	Compatible with Dimethylformamide
2.0 ml screw-top polypropylene tubes	Sarstedt	72694005	Compatible with Dimethylformamide
50 ml tubes	BD Falcon	352070	Compatible with Dimethylformamide
Agencourt RNAClean XP	Beckman Coulter	A63987	We recommend to use these paramagnetic beads. Aliquot and store at 4°C
Chloroform	Sigma Aldrich	372978	WARNING – HAZARDOUS TO HEALTH
Dimethylformamide	Sigma Aldrich	D4551	
Dithiothreitol (DTT)	Roth	6908.1	Prepare as 100 mM DTT in nuclease-free H ₂ O; always prepare fresh
Ethanol	Merck	1.00983.1000	
EZ-Link Biotin-HPDP (50 mg)	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. DMF dissolves some plastic materials. We recommend to use glass pipettes to transfer DMF from ist stock glass bottle to 50 ml Falcon tubes.
High Sensitivity DNA Kit	Agilent Technologies	5067-4626	
Isopropanol	Merck	1.09634.1011	
NaCl (5M)	Sigma Aldrich	71386	Stock solution
nuclease-free EDTA (500 mM), pH 8.0	Invitrogen	15575-020	Stock solution

Nuclease-free H ₂ O	Sigma Aldrich	W4502	Stock solution
nuclease-free Tris Cl (1M), pH 7.4	Lonza	51237	Stock solution
Phase Lock Gel Heavy tubes (2.0 ml)	5Prime	2302830	Use in step 1.3.4.
Polypropylene 15 ml centrifuge tubes	Greiner Bio-One	188271	Or equivalent; they have to tolerate up to 15,000 × g
QIAzol Lysis Reagent (200 ml)	Qiagen	79306	Use this or equivalent TRI reagent for RNA isolation, WARNING – CORROSIVE and HAZARDOUS TO HEALTH! Ensure immediate access to Phenol antidote (PEG-Methanol)
Qubit RNA HS assay kit	Life Technologies	Q32852	Use this kit for quantifying RNA quantity in step 1.4.11
RNeasy MinElute Kit	Qiagen	74204	Optional; includes Buffer RLT
Sodium citrat	Sigma Aldrich	C8532	Prepare 1.6 M stock solution using nuclease-free water
Tween 20	Sigma Aldrich	P1379	
μMacs Streptavidin Kit	Miltenyi	130-074-101	Store at 4°C, includes μMacs columns used in step 1.4.6. (store at RT)
Cell viability and stress assay			
PE Annexin V Apoptosis Detection Kit I	BD Biosciences	559763	Optional
Thapsigargin	Sigma-Aldrich	T9033	Optional
p53	abcam	ab26	Optional
p-EIF2α (Ser51)	Cell Signaling	9721	Optional
BH3I-1	Sigma-Aldrich	B 8809	Optional
Buffers			
4sU Washing Buffer		store at RT	100 mM Tris pH 7.4, 10 mM EDTA, 1 M NaCl, 0.1% Tween 20 in nuclease-free H ₂ O
Biotinylation Buffer (10x)		store at 4 °C	100 mM Tris pH 7.4, 10 mM EDTA in nuclease-free water; make aliquots of 1 ml; store at 4°C
RNA precipitation buffer		store at RT	1.2 M NaCl, 0.8 M sodium citrate in nuclease free water. Prepare in advance under nuclease-free conditions. Store at room temperature in 50 ml falcon tubes.
Equipment			
2100 Bioanalyzer instrument	Agilent	G2939BA	
RNA 6000 Nano Kit	Agilent	5067-1511	Use this kit to verify RNA integrity in step 1.3.10
RNA 6000 Pico Kit	Agilent	5067-1513	Optional
UV/VIS spectrophotometer	Thermo Scientific	NanoDrop 1000	Or equivalent. Use in step 1.2.2/3.1.8/3.3.3/3.4.3
High-speed centrifuge	Thermo Scientific	Heraeus Multifuge X3R	Or equivalent equipment capable of reaching 13,000 × g
High-speed rotor	Thermo Scientific	Fiberlite F15-6 x 100y	
Adaptors for 15 ml tubes			
Refrigerated table-top centrifuge	Eppendorf	5430 R	Or equivalent.
Thermomixer	Eppendorf	Thermomixer C	Or equivalent.

Magnetic stand	Miltenyi Biotec	130-042-109	One stand holds 8 μ Mac columns.
Ultra-fine scale	Mettler Toledo	ML204T	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.
DynaMag-2 Magnet-1 each	Life Technologies	12321D	
RNaseZap	Sigma	R2020	Optional
TruSeq stranded total RNA library prep kit	Illumina	RS-122-2201	Or equivalent. For T cells we used 400 ng 4sU and Total RNA with 11 cycles for PCR amplification. rRNA depletion is included in this kit
Nanodrop	Thermo Scientific		use a Nanodrop or equivalent instrument to measure RNA concentration
Ribosome Profiling			
TruSeq Ribo Profile kit (Mammalian or Yeast)	Illumina	RPYSC12116 (Yeast)	
TruSeq Ribo Profile kit (Mammalian or Yeast)	Illumina	RPHMR12126 (Mammalian)	
Illustra MicroSpin S-400 HR Columns	GE Healthcare	27-5140-01	
RNA Clean & Concentrator-25 kit	Zymo Research	R1017	
RNA Clean & Concentrator-5 kit	Zymo Research	R1015	
Ribo-Zero Gold rRNA Removal Kit (Human/Mouse/Rat)	Illumina	MRZG126 or MRZG12324	
(High Sensitivity DNA Kit)	Agilent Technologies	5067-4626	Already needed for 4sU-seq
All other consumables and equipment are listed in the User guide	!!!		Carefully read the user guide and order required consumables in advance (consider a long delivery time for some consumables e.g. gels)
ChIP			
10 mM Tris-HCl (pH 8.0)	general lab supplier		
100 bp Plus Marker	Thermo Fisher	SM0323	
16 % Formaldehyde	Thermo Fisher	28908	Add to a final concentration of 1 %
70% EtOH	general lab supplier		Always prepare fresh
Agarose	general lab supplier		
Agencourt RNAClean XP beads	Beckman Coulter	A63987	We recommend to use these paramagnetic beads. Aliquot and store at 4°C
ChIP library preparation kit	KapaBiosystems	KK8504	Or use the kit of your choice
DNA low bind microcentrifuge tubes	Eppendorf	Z666548-250EA	or equivalent
Dynabeads Protein G	Invitrogen	10004D	Use these superparamagnetic beads coupled to protein G in step 4.3.1.; Bring to RT before use
Glycine	general lab supplier		Prepare a 2M stock solution
Glycogen	Roche	10-901-393-001	
MinElute PCR Purification Kit	Qiagen	28004	Use this kit (or equivalent) to purify chromatin in step 4.2.4.
Phosphatase Inhibitor (PhosStop)	Roche	4906837001	Add freshly to the buffer and keep on ice
Power SYBRgreen Master mix	Thermo Fisher	4367659	

Protease Inhibitor (cOmplete, EDTA-free)	Roche	11873580001	Add freshly to the buffer and keep on ice
Proteinase K	Invitrogen	25530049	
Qubit dsDNA HS Assay kit	Invitrogen	Q32851	Use this kit for quantifying DNA quantity in step 4.6.4. on a Qubit Fluorometer
Rnase, DNase free	Roche	11-119915001	
Salmon sperm (sonicated to around 100bp)	Sigma	D1626	
TE pH 8.0	general lab supplier		
Antibodies (ChIP grade if possible)			
anti-RNA Pol II [8WG16]	abcam	ab817	
anti-Histon H3K36me3	abcam	ab9050	
or antibody of interest			
Buffers			
Binding/Blocking buffer		Store at RT	PBS with 0.5 % BSA and 0.5 % Tween 20
Cell-Lysis buffer		Store at RT	5 mM Pipes [pH 8.0], 85 mM KCl, and 0.5 % NP40
ChIP IP buffer		Store at RT	0.01 % SDS; 1. 1% Triton X-100; 1.2 mM EDTA; 16.7 mM Tris-HCl, pH 8.1; 16.7 mM NaCl
Elution buffer		Store at RT up to 6 months	10 mM Tris-HCl (pH 8.0), 5 mM EDTA (pH 8.0), 300 mM NaCl and 0.5 % SDS
Nuclei-Lysis buffer		Store at RT	50 mM Tris [pH 8.0], 10 mM EDTA, and 1 % SDS
Wash buffer I		Store at RT	0.1 % SDS; 1 % Triton X-100; 2 mM EDTA; 20mM Tris-HCL pH 8.1; 150 mM NaCl
Wash buffer II		Store at RT	0.1 % SDS; 1 % Triton X-100; 2 mM EDTA; 20 mM Tris-HCL pH 8.1; 500 mM NaCl
Wash buffer III		Store at RT	0.25 M LiCl; 1% NP-40; 1 mM EDTA; 10 mM Tris-HCl, pH 8.1
Equipment			
2100 Bioanalyzer instrument	Agilent	G2939BA	use this instrument for electrophoretical analysis
Nanodrop	Thermo Scientific		
Bioruptor TBX microtubes 1.5 ml	Diagenode	C30010010	
or tubes special for your sonication device			
Bioruptor sonication device or sonication device of your choice			Sonication of T cells with Bioruptor: 20 - 25 cycles (30 s on, 30 s off at high in two 1.5 ml bioruptor microtubes with 500 µl each tube)
Magnetic stand for tubes			
Thermomixer			
Agarose gel electrophoresis			
Qubit Fluorometer	Thermo Scientific		Use this Fluorometer for quantifying low amounts of RNA/ DNA