Nuclear Run On Transcription Assay

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Abstract

Adapted from Core LJ, Waterfall JJ, Lis JT. Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science. 2008;322(5909):1845-8. PubMed PMID: 19056941.

This method can be used to detect nascent transcripts. The conditions given are suitable for HeLa cells and should be adapted if other cell types are used.

Citation: Xavier Contreras,Rosemary Kiernan Nuclear Run On Transcription Assay. **protocols.io** dx.doi.org/10.17504/protocols.io.khxct7n
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Guidelines

This protocol is for 1 confluent 15 cm^2 dish of HeLa cells. Parameters should be adapted for other cell types.

Before start

Prepare the following reagents in advance and store at +4°C:

Swelling buffer:	**Stock**	**Final Conc**	**Vol (for 50 ml)**
Tris-HCl, pH 7.5	1 M	10 mM	500 µl
$MgCl_2$	1 M	2 mM	100 µl
$CaCl_2$	1 M	3 mM	150 µl
Water			to 50 ml

Lysis Buffer:	**Stock**	**Final Conc**	**Vol (for 50 ml)**
Swelling buffer +			
Igepal	50 %	0.5 %	500 µl
Glycerol	100 %	10 %	5 ml
RNasin	40 U/µl	2 U/ml	100 U
Water			to 50 ml

Freezing Buffer:	**Stock**	**Final Conc**	**Vol (for 50 ml)**
Tris-HCl pH 8.3	1 M	50 mM	2.5 ml
Glycerol	100 %	40 %	20 ml
$MgCl_2$	1 M	5 mM	250 µl
EDTA	0.5 M	0.1 mM	10 µl
Water			to 50 ml

Assay Buffer:	**Stock**	**Final Conc**	**Vol (for 5 ml)**
Tris-HCl pH 8.0	1 M	10 mM	50 µl
MgCl2	1 M	5 mM	25 µl
DTT	0.1 M	1 mM	50 µl
KCl	3 M	300 mM	0.5 µl
RNasin	40 U/µl	20 U/ml	2.5 µl
*Sarkosyl	10 %	1 %	500 µl
ATP, GTP, CTP and UTP/Br-UTP		500 µM	
Water			to 5 ml
*Add sarkosyl just before starting incubation			

Binding Buffer for anti BrdU:	**Stock**	**Final Conc**	**Vol (for 14 ml)**
SSPE	20 x	0.25 x	175 µl
EDTA	0.5 M	1 mM	28 µl
Tween20	10 %	0.05 %	70 µl
NaCl	5 M	37.5 mM	105 µl
Water			to 14 ml

Blocking Buffer:	**Stock**	**Final Conc**	**Vol (for 2 ml)**
Binding Buffer + polyvinylpyrrolidone (PVP)	10 %	0.1 %	20 µl
BSA	50 µg/ml	1 µg/ml	40 µl
Water			to 2 ml

Low Salt Wash Buffer:	**Stock**	**Final Conc**	**Vol (for 10 ml)**
SSPE	20 x	0.2 x	100 µl
EDTA	0.5 M	1 mM	20 µl
Tween20	10 %	0.05 %	50 µl
Water			to 10 ml

High Salt Wash Buffer:	**Stock**	**Final Conc**	**Vol (for 10 ml)**
SSPE	20 x	0.25 x	125 µl
EDTA	0.5 M	1 mM	20 µl
Tween20	10 %	0.05 %	50 µl
NaCl	5 M	100 mM	200 µl
Water			to 10 ml

Elution Buffer :	**Stock**	**Final Conc**	**Vol (for 5 ml)**
DTT	1 M	20 mM	100 µl
NaCl	5 M	150 mM	150 µl
Tris-HCl pH 7.5	1 M	50 mM	250 µl
EDTA	0.5 M	1 mM	10 µl
SDS	20 %	0.1 %	25 µl
Water			to 5 ml

TET:

Tris.EDTA + 0.5 % Tween20

Add 2 µl of RNasin to all buffers (40 U/µl) unless otherwise stated

Materials

brdU/BrU conjugated beads SCBT-32323Ac by Santa Cruz Biotechnology

- ATP 035RA02825 by Contributed by users
- CTP 035RC02825 by Contributed by users
- GTP 035RG02825 by Contributed by users

RNasin by Promega

sarkosyl L5777 by Sigma Aldrich

sspe 20x by Sigma

Protocol

Isolation of nuclei

Step 1.

Wash cells 3 times directly in 15 cm^2 dish with ice cold PBS

Add 10ml of ice cold swelling buffer directly onto the cells and incubate on ice for 5 min

Scrape cells and transfer to a 15 ml conical tube and pipette up and down several times with cut-off pipette tips

Centrifuge at 4°C, at 1000 rpm for 10 min

Remove supernatant, add 1 ml of lysis buffer and pipette up and down several times with cut-off pipette tips (pellet contains isolated nuclei)

Make the volume up to 10ml with buffer

Wash nuclei once with lysis buffer

Resuspend in 1 ml of freezing buffer

Centrifuge at 1000 xg for 10 mins

Resuspend in 200 µl of freezing buffer (It will be a bit difficult to resuspend, so use cut-off tips)

Run on transcription reaction

Step 2.

Divide the samples into two tubes, one for BrUTP and one for UTP

Add 100 µl of Assay Buffer (assay Buffer contains all NTPs except BrUTP/UTP)

*Add Sarkosyl to individual tubes (20 µl of 10% stock/200 µl reaction) after the run on reaction buffer is added

Add BrUTP/UTP

Incubate the reaction at 31°C for 15 min with shaking every 5 min

RNA purification

Step 3.

Extract RNA with Trizol by adding 0.8 ml of Trizol to 200 µl run on reaction

Pipette up and down with tips

Add chloroform and precipitate with isopropanol (as for Trizol extraction)

Dissolve RNA with 90 µl water, incubate at 65°C for 5min

Add 10 µl 10x DNAse buffer

Add 2 µl DNAse1

Incubate for 20 min at 37°C (in a thermomixer if possible)

Heat inactivate DNase (65°C for 10 min or according to manufacturer's instructions)

Add 100 µl water, 300 mM NaCl, 200 µl isopropanol

Centrifuge 15 min at 13x g

Wash with 70% ethanol

Dissolve RNA in 50 µl DEPC water

Immunoprecipitation

Step 4.

Equilibrate 40 µl of 25% slurry of BrdU/BrU conjugated beads in 500 µl binding buffer 2x

Add buffer, rotate tubes for 5 min on a rotating wheel (8 rpm) and centrifuge at 1000 x g for 2 min
Place on ice for 1 min before removing the supernatant

Block the beads in 5x volume of blocking buffer for 2 hours at 4°C

(Add extra 2 µl RNasin for every 1 ml of blocking buffer at this step)

Wash 2x with 500 µl of binding buffer

Resuspend beads in 400 µl binding buffer/ reaction

Bring NRO RNA to 100 µl and heat sample at 90°C for 5 min then place on ice for 2-3 mins, add 300 µl binding buffer, add 5 mM EDTA

Bind RNA to beads for 1 h* at 4°C. (* test times between 30 mins and 2 h)

Perform washes as follows: rotate tube for 3-5 min on a rotating stand, centrifuge at 1000 x g for 3 min, discard the supernatant

Wash 1x in 500 µl binding buffer

Wash 1x in 500 µl low salt buffer

Wash 1x in 500 µl high salt buffer

Wash 2x in 500 µl TET buffer

Elute 4x in 125 µl of elution buffer that has been heated to 42°C. Vortex the tubes every few minutes

Perform acid phenol chloroform extraction once

Add 1 µl glyco blue

Add 0.3 M (final conc) of ammonium/sodium acetate (stock 5 M)

Add 3 vol of 100% ethanol

Precipitate at -20°C for 20 min

Spin and wash with 70% ethanol

Resuspend in 30 µl of DEPC water

Quantify by nanodrop