Protocol	#8.1
Title	**BOMB total RNA extraction from mammalian cells using TRI reagent**
Keywords	HT RNA isolation, Silica-beads, TRI reagent
Authors	Oberacker P*, Stepper P*, Bond DM*, Höhn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA# and Jurkowski TP#
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Summary

Piotr Chomczyński and Nicoletta Sacchi developed the rapid single-step TRI protocol for RNA isolation in 1987 [1]. This protocol has since been used extensively to purify high quality RNA [2]. It employs acid guanidinium-thiocyanate and phenol to lyse cells and inactivate proteins including RNases. Here, we combine it with capturing the RNA on silica-coated magnetic beads instead of centrifugation and phase separation followed by DNase I treatment and RNA clean-up. If the DNase digest step is omitted, TNA can be isolation (see BOMB protocol #6.2).

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
$CaCl_2$	Roth Chemicals	CN92.2	110.99	 Danger	H: 302-319-373 P: 260-264-270-280-314-330-337+313-501
DNase I	Biozym	170500	n.a.	n.a.	n.a.
Ethanol (C_2H_6O, 99.9%)	Honeywell/Riedel-de Haën	34963	46.07	 Danger	H: 225-319 P: 210-240-305+351+338-403+233
Guanidinium chloride (GuHCl, $CH_5N_3 \cdot HCl$)	Roth Chemicals	0037.1	95.53	 Attention	H: 302+332-315-319 P: 261-280-301+312-330-304+340+312-305+351+338-337+313
$MgCl_2$	Roth Chemicals	2189.1	203.36	n.a	n.a.
RNasin	Promega	N2515	n.a.	n.a.	n.a.
TRI reagent	Refer to #B BOMB TRI reagent protocol		n.a.	 Danger	H: 301+311+331-314-335-341-373-412 P: 201-261-264-280-273-301+310-302+352-303+361+353-304+340-305+351+338
Tween 20	Roth Chemicals	9127.1	1228.	n.a.	n.a.

Please consult appropriate MSDS information before working with these chemicals! Use lab coat, gloves and eye protection at all times! The chemicals are available from other providers as well. No preference is given to the indicated vendors.

Buffers and solutions

TRI reagent or **TRIzol** (200 µl per sample, 20 ml per 96-well plate)

90% ethanol (3.2 ml per sample, 320 ml per 96-well plate)

RNA binding buffer (0.8 ml per sample, 80 ml for 96-well plate, adjust pH to ~6)

Reagent	Concentrations	For 100 ml
Gu-HCl	1 M	9.55 g
Tween 20	0.05%	0.5 ml of 10% stock
Ethanol	~ absolute	100 ml of 99.9% stock

10x DNase I buffer (prepare 50 ml, aliquot and freeze)

Reagent	Final concentration	For 50 ml
Tris-HCl pH 7.6	100 mM	5 ml of 1 M stock
Tween 20	0.5%	2.5 ml of 10% stock
$MgCl_2$	25 mM	
$CaCl_2$	5 mM	

adjust pH with HCl/NaOH to pH ~7.6 and adjust the volume with water to 50 ml

DNase I reaction mix (150 µl per sample, 15 ml for one plate. Prepare fresh and keep on ice)

Reagent	Final concentration	For a 96-well plate
10x DNase I buffer	1x	1.5 ml of 10x stock
DNase I (1000 U/ml)	20 U/ml	300 µl 1000 U/ml stock
RNasin	0.05%	7.5 µl of stock
Nuclease free water (e.g. DEPC-treated)		13.2 ml

Equipment and setup

Fume hood

Temperature controlled incubation shaker or incubator (e.g. Infors HT Multitron Pro)

Plate centrifuge with swing-out rotor (e.g. Eppendorf Centrifuge 5804R)

Heat block (e.g. Roth, Rotilabo®-block thermostat H250 – PN: Y264.1)

Multistep pipette with 100 µl tips (e.g. Eppendorf, Multipette® M4 – PN: 4982000012)

Microtiter plate orbital shaker (e.g. IKA MS 3 basic)

Magnetic stand for 96-well plate (e.g. BOMB microplate magnetic rack)

Multichannel Pipettes

10 - 100 µl (e.g. Eppendorf, Eppendorf Research® plus 8-Channel – PN: 3122000035)

30 - 300 µl (e.g. Eppendorf, Eppendorf Research® plus 8-Channel – PN: 3125000052)

50 - 1200 µl (e.g. VWR, Multi-channel pipette, 8-channel – PN: 613-5422)

1.2 ml 96-well deep well plates (e.g. Sarstedt, MegaBlock® 96 Well – PN: 82.1971.002)

96-well PCR plate (e.g. Sarstedt, 96 PCR-Plate half skirt flat – PN: 170134)

Seals (e.g. Bio-Rad, Microseal® 'B' Adhesive Seals – PN: MSB1001)

BOMB Total RNA extraction using TRI

Step	Sample preparation	Time	☑
1	Collect up to 10^6 cultured mammalian cells in a single well of a deep-well plate		☐
2	Pellet the cells via centrifugation at 500g and discard the supernatant	5 min	☐
	Opt *At this point, the pellets can be frozen at -80 °C and processed later*		
3	Resuspend and lyse the cells in 200 µl **TRI reagent** (TRIzol or similar) at 1300 rpm for 15 min (or until dissolved) *Phenol (in TRIzol) is highly toxic; perform all manipulations with personal protective equipment. Always use a fume hood. Dispose phenol-containing trash following local requirements!*	30 min	☐
	Opt *At this point, the plates can be sealed and kept at 4 °C overnight or frozen at -20 °C and processed later*		
Step	**RNA purification**		
4	Add 200 µl of **RNA binding buffer**	5 min	☐
5	Add 40 µl **silica-coated magnetic beads** (BOMB protocol #2.1, 1:10 diluted from stock) and shake for 5 min at 1100 rpm	10 min	☐
6	Settle the magnetic beads on a magnetic stand, remove and discard the cleared supernatant. Aspirate the solution slowly to avoid losing the beads	10 min	☐
Opt	*We recommend an additional wash step with 200 µl **TRI reagent** and 200 µl **RNA binding buffer** when isolating from sources with a high content of RNases*	10 min	
7	Remove the plate from the magnetic stand, add 400 µl **90% ethanol** and mix well	5 min	☐☐ ☐☐
8	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐☐ ☐☐
9	**Repeat** steps **7-8 three** more times for a total of **four washes**	15 min	☐
10	Remove the supernatant completely and dry the beads at 50 °C for ~5 min to evaporate ethanol traces	5 min	☐
11	Add 150 µl of **DNase I reaction mix** and mix at 1300 rpm for 5 min at RT, centrifuge shortly and shake at 350 rpm for 15-60 min at 37 °C	60 min	☐
12	Add 600 µl **RNA binding buffer** to the digest and mix at 1000 rpm for 10 min	15 min	☐
13	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐
14	Add 400 µl **90% ethanol** and mix well	5 min	☐☐ ☐☐
15	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐☐ ☐☐
16	**Repeat** steps **14-15 three** more times for a total of **four washes**	10 min	☐
17	Dry the beads on a heat block at 50 °C for ~30 min *The beads are dry when they turn brownish. Do not elute earlier!*	45 min	☐
18	Add 40 µl of **nuclease-free water** to elute RNA, mix at 1300 rpm for 5 min	10 min	☐
19	Pellet the magnetic beads on a magnetic stand and transfer the eluted RNA to a fresh 96-well collection plate	10 min	☐
End	Measure concentration and purity	**~3.5 h** (1.5 h hands-on)	
	Store @ -80 °C		

Troubleshooting

Problem	Solution
Beads sticking to the sides	• Sonicate shortly in the sonic bath and/or push the beads down with a pipette tip
Beads stay at the bottom of the well when mixing	• Use a good foil to seal the plate well and invert a few times
Incomplete lysis	• Resuspend the pellets by pipetting if they did not dissolve by shaking
DNA contamination	• If some beads are still above the water level before or during the DNase digest, push them down with a clean yellow pipette tip
Degraded RNA	• Process samples immediately after collection • Perform the additional wash step with TRI reagent as recommended for sources with high content of RNases
TRI reagent spillage	• Have a bottle of PEG 300 or 400 nearby and use this to wipe off immediately and repeatedly

Exemplary Results

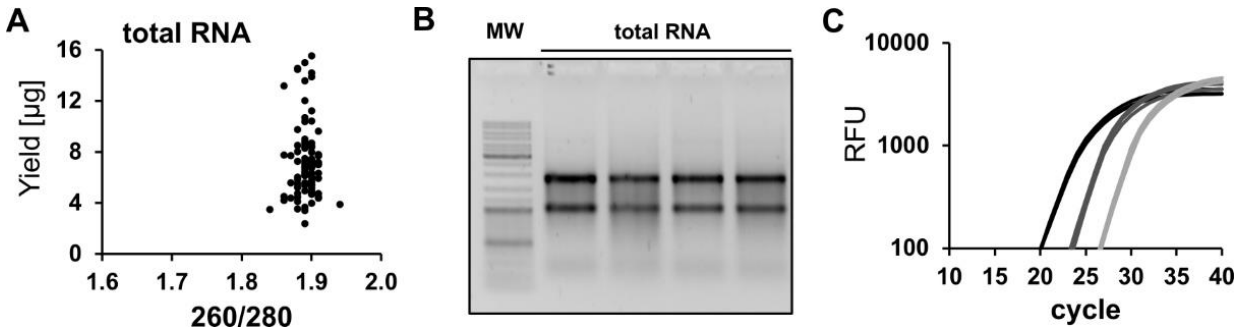

Fig 1: Quality control of BOMB total RNA extraction using TRI-reagent from cultured cells. *(A) Total yield of extracted total RNA from 0.5 million* HEK293 *cells, plotted over A260 nm/A280 nm ratio. (B) TAE agarose gel of five samples after isolation of RNA. 300 ng were loaded per sample. MW: GeneRuler DNA Ladder Mix, Thermo Scientific. (C) qPCR amplification curve of a 10-fold serial dilution (black: undiluted, dark grey: 1:10 diluted, light grey: 1:100 diluted). RNA was isolated from HEK293 cells and reverse-transcribed into cDNA.*

References

1. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium extraction by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162: 156–159. doi:10.1016/0003-2697(87)90021-2

2. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twenty-something years on. Nat Protoc. 2006;1: 581–585. doi:10.1038/nprot.2006.83