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|---|---|
| **Protocol** | #8.2 |
| **Title** | **BOMB total RNA extraction from mammalian cells using GITC-lysis** |
| **Keywords** | HT RNA isolation, carboxyl-beads, silica beads |
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| **Citation** | Oberacker et al.(2019), Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid manipulation. PLOS Biology,17(1), https://doi.org/10.1371/journal.pbio.3000107 |
| **Online** | https://bomb.bio/protocols/ |
| **Revision** | V1.0 (13th August 2018) |

## Summary

The most popular technique for isolation of high quality RNA (that does not use columns) is the TRI method[1,2]. However, the need for highly toxic phenol makes the procedure dangerous and requires you to use a fume hood. Here, we present a method for isolation of total RNA from cells with magnetic beads based on lysis and RNase inactivation by guanidine isothiocyanate (GITC) [3]. Thanks to an on-bead DNase I digest, it delivers highly pure RNA (or TNA if you skip the DNase I step, as in BOMB protocol #6.1).

## Chemicals

| Name | Provider | PN | MW [g/mol] | Safety codes | |
|---|---|---|---|---|---|
| **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 |
| **Guanidine isothiocyanate (GITC, $C_2H_6N_4S$)** | Roth Chemicals | 2628.4 | 118.16 |  Warning | H: 302+312+332-412-EUH032 P: 273-280-302+352-304+340-312 |
| **Tris(hydroxymethyl)-aminomethane (Tris, $C_4H_{11}NO_3$)** | Roth Chemicals | AE15.3 | 121.14 |  Warning | H 315-319-335 P: 280-302+352-305+351+338-312 |
| **N-Lauroylsarcosine sodium salt (Sarkosyl, $C_{15}H_{29}NO_3Na$)** | Sigma (Merck) | L9150-50G | 293.38 |  Danger | H: 315-318-330 P: 260-280-284-305+351+338-310 |
| **Antifoam 204** | Sigma (Merck) | A8311-50ML | n.a. | n.a. | n.a. |
| **Ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA ($C_{10}H_{14}N_2Na_2O_8 \cdot 2\ H_2O$)** | Roth Chemicals | 8043.1 | 372.24 |  Danger | H: 332-373 P: 260-314 |
| **Isopropanol ($C_3H_8O$)** | Acros Organic | 184130025 | |  Danger | H: 225-319-336 P: 210-233-240-305+351+338-403+235 |
| **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 |
| **Tween 20** | Roth Chemicals | 9127.1 | 1228. | n.a. | n.a. |
| **DNase I** | Biozym | 170500 | n.a. | 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

**Silica-coated magnetic beads** (40 µl per sample, 4 ml for a 96-well plate)

**Lysis buffer** (stable for at least several weeks at RT. 240 µl per sample, 24 ml per 96-well plate)

| Reagent | Concentrations | For 50 ml |
|---|---|---|
| **GITC** | 4 M | 23.64 g |
| **Tris HCl pH 7.6-8.0** | 50 mM | 2.5 ml of 1 M stock |
| **Sarkosyl** | 2% | 1 g |
| **EDTA** | 20 mM | 2 ml of 0.5 M stock |
| **Antifoam** (optional) | 0.1 % | 50 µl |

adjust pH with HCl to 7.6-8.0 and adjust the volume with water to 50 ml

**Isopropanol** (720 µl per sample, 72 ml for a 96-well plate)

**80% ethanol** (3.2 ml per sample, 220 ml for a 96-well plate)

**RNA binding buffer** (0.6 ml per sample, 60 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)

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

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

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

**Multichannel Pipettes**

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)

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

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

**Reservoirs** (e.g. Roth, Rotilabo®-liquid reservoirs – PN: E830.2)

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

# BOMB total RNA from mammalian cells extraction using GITC

| Step | Sample collection | Time | ☑ |
|---|---|---|---|
| 1 | Collect up to $10^6$ cultured mammalian cells a single well of a deep-well plate | | ☐ |
| 2 | Pellet the cells via centrifugation at 500g and discard supernatant | | ☐ |
| | *At this point, the pellets can be frozen at -20 °C and processed later* | | |
| **Step** | **RNA purification** | | |
| 3 | Add 240 µl of **lysis buffer**, seal and shake at RT at 1400 rpm for 5 min | | |
| ⚠ | *The lysis buffer contains guanidine isothiocyanate, a chemical which can cause burns and sensitivity and produces hydrogen cyanide when mixed with bleach/acids* | 10 min | ☐ |
| 4 | Add 320 µl of **isopropanol**, seal and shake at RT at 1400 rpm for 5 min | 10 min | ☐ |
| 5 | Add 40 µl of **silica-coated magnetic beads (**BOMB protocol #2.1, 1:10 diluted from stock), seal and shake at RT at 1400 rpm for 5 min | 10 min | ☐ |
| 6 | Settle the magnetic beads on a magnetic stand and discard the supernatant | 5 min | ☐ |
| ⚠ | *Ensure that the beads are completely settled* | | |
| 7 | Remove the plate from the magnetic stand and add 400 µl **isopropanol**. Shake at RT at 1400 rpm for 2 min | 10 min | ☐ |
| 8 | Settle the magnetic beads on a magnetic stand and discard the supernatant | 5 min | ☐ |
| 9 | Add 400 µl **80% ethanol** and mix well | 5 min | ☐☐ ☐☐ |
| 10 | Settle the magnetic beads on a magnetic stand and discard the supernatant | 5 min | ☐☐ ☐☐ |
| 11 | **Repeat** steps **9-10 three** more times for a total of **four washes** | 10 min | ☐ |
| 12 | Remove the supernatant completely and dry the plate at 50 °C for ~ 5-10 min | 5 min | ☐ |
| ⚠ | *Make sure to remove all remaining ethanol.* | | |
| 13 | Remove the plate from the magnets and 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 | 30 min | ☐ |
| 14 | Add 600 µl **RNA binding buffer** to the digest and mix at 1000 rpm for 10 min | 15 min | ☐ |
| 15 | Settle the magnetic beads on a magnetic stand and discard the supernatant | 5 min | ☐ |
| 16 | Add 400 µl **80% ethanol** and mix well | 5 min | ☐☐ ☐☐ |
| 17 | Settle the magnetic beads on a magnetic stand and discard the supernatant | 5 min | ☐☐ ☐☐ |
| 18 | **Repeat** steps **16-17 three** more times for a total of **four washes** | 10 min | ☐ |
| 19 | Dry the beads on a heat block at 50 °C for ~30 min | 30 min | ☐ |
| 👁 | *The beads are dry when they turn brownish. Do not elute earlier!* | | |
| 20 | Add 40 µl of **nuclease-free water** to elute RNA, mix at 1300 rpm for 5 min | 10 min | ☐ |
| 21 | 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 | **~3.0 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 cover the plate well and invert a few times |
| **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<br>• Perform the additional wash step with TRI reagent as recommended for sources with high content of RNases |

## Exemplary results

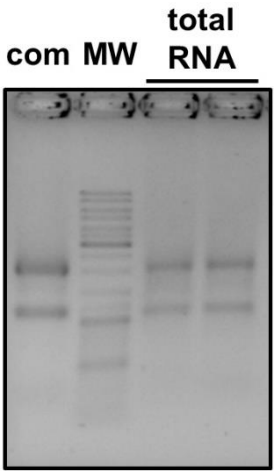


***Fig 1:*** **Quality control of BOMB total RNA extraction.** Representative agarose gel of isolated RNA from 500k HEK293 cells. The leftmost lane contains RNA extracted with a commercial column-based kit, whereas the two lanes on the right contain RNA extracted with the BOMB total RNA isolation protocol based on the GITC. MW: GeneRuler DNA Ladder Mix, Thermo Scientific.

## 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

3. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 1979;18: 5294–5299. doi:10.1021/bi00591a005