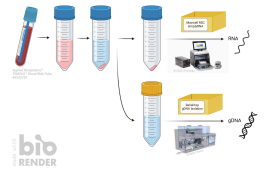




Jun 27, 2025

RNA and gDNA Isolation from stabilized Whole Blood (Tempus Blood RNA Tube) using semi-automated Promega Chemistry



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We use this protocol and it's working

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Funders Acknowledgements:

Disclaimer

All authors declare no conflicts of interest. There have not been any financial incentives for the publication of this protocol.

While we believe that, a similar workflow can be adapted for different manufacturers liquid-handlers, this protocol in its published form has only been validated on machines and chemicals as described here.

Abstract

Here we describe a semi-automated way to isolate RNA and gDNA from (the same) TEMPUS Tube. This allows the user to expand the use of TEMPUS Tubes (designed for RNA stability only) as well as describing a high throughput, highly reproducible, semi-automated way using liquid handlers in the isolation process.

Attachments



[4379232_TempusTubesI](#)

...

548KB



[Maxwell RSC](#)

[simplyRN...](#)

283KB



[AN186 Isolating gDNA...](#)

126KB

Image Attribution

Merz MP, created with BioRender



Guidelines

The Central Biobank Charite (ZeBanC) works under the best practice laboratory guidelines and ISO:9001 quality standards.

This protocol uses readily available Kits (by PromegaTM) in a semi-automated fashion. While we believe that, a similar workflow can be adapted for different manufacturers liquid-handlers, this protocol in its published form has only been validated on machines and chemicals as described here.

In case of doubt, please feel free to consult attached manufacturers SOPs or reach out to us.

Materials


 Maxwell® RSC simplyRNA Blood Kit
 Promega Catalog #AS1380


 ReliaPrep™ Large Volume HT gDNA Isolation System
 Promega Catalog #A2751


 TempusTM Blood RNA Tube
 Thermo Fisher Scientific Catalog #4342792

Equipment

Falcon® Centrifuge Tubes

NAME

Polypropylene, Sterile, 50 mL

TYPE

Corning®

BRAND

352070

SKU

Equipment

centrifuge tube, 50mL

NAME

50 mL conical bottom tube; polypropylene

TYPE

TPP® centrifuge tubes

BRAND

TPP 91051

SKU

https://www.tpp.ch/page/produkte/13_zentrifugen_roehrchen.php
LINK

Equipment

Maxwell RSC instrument	NAME
Automated nucleic acid purification platform	TYPE
Promega	BRAND
AS4500	SKU

Equipment

Freedom EVO-2 HSM2 (custom)	NAME
liquid handling and robotics workstation	TYPE
TECAN (RRID:SCR_016771)	BRAND
30084252	SKU
https://lifesciences.tecan.com/freedom-evo-platform	LINK

Protocol materials


 Maxwell® RSC simplyRNA Blood Kit **Promega Catalog #AS1380**


 ReliaPrep™ Large Volume HT gDNA Isolation System **Promega Catalog #A2751**


 Tempus™ Blood RNA Tube **Thermo Fisher Scientific Catalog #4342792**



Before start

Materials to Be Supplied by the User:

- vortex mixer
- 50ml tubes (sterile)
- centrifuge with swinging-bucket rotor
- RNase-free, sterile, aerosol-resistant pipette tips
- Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)
- ReliaPrep™ Large Volume HT gDNA Isolation System (Cat.#A2751)

Blood draw, tube handling and storage

- 1 Draw up to **3 mL of blood directly into a Tempus™** Blood RNA Tube according to your laboratory's or hospital standard. See manufacturers SOP for more information.

10m



[picture from thermofisher.com](https://www.thermofisher.com)

Note

Beware of underfilling the Tempus tube (black mark on tube). In our experience, underfilling is the biggest contributor to a noticeable decrease of the yield and quality of RNA and gDNA.

- 2 Immediately after filling the tube, shake the tube vigorously or vortex the contents for 10 sec to ensure that the stabilizing reagent is thoroughly mixed with the sample.

Note

In our experience, inverting the tube several times (comparable to other blood sampling tubes) is sufficient for nucleic acid stabilization. However this is not in line with manufacturer instructions.

- 3 Filled Tempus tubes should be shipped at room temperature (18-25°C) within a maximum of 5 days. Please consult manufacturer instructions for details.
- 4 Filled Tempus can be frozen and stored at -20°C to -80°C until nucleic acid isolation.



Note

At ZeBanC, we freeze Tempus tubes at -20°C overnight and subsequently transfer them to -80°C the next day for long-term storage. This has merely practical reasons for our internal workflow.

We have already isolated good quality RNA & gDNA from tubes stored (@ -80°C) for up to 6 years.

RNA isolation from Tempus tubes

5 Solution Preparation

15m

This can be done in advance. **Handle in fume hood!**

Prepare (1-Thioglycerol) Homogenisation buffer (we do it for a batch of 32 samples)

🧴 12 mL Homogenisation Solution (Promega #Z305H)

🧴 240 µL 1-Thioglycerol (Promega #A208B)

Stable at 2-10°C for up to 30 days.

Prepare Rehydrate DNase-I solution (28 samples/tube) according to the manual.

Dissolve lyophilysed DNase-I in 🧴 275 µL nuclease-free-water and add

🧴 5 µL Blue-Dye

Should be stored at -20°C until use. Avoid repeated thaw-freeze cycles.

6 Thaw (32) Tempus tubes at room temperature (20-22°C). This will take approximately 30-60min.

1h

Note

Do NOT exceed 25°C. Thawing in a water bath did not improve handling and/or yield for us.

Alternatively, samples can be thawed at 4°C over night. We have found no difference in quality or yield between when thawing at 4°C overnight (compared to 60 min @RT) and find handling quite convenient.

We handle 32 samples at a time, since this is the maximum our (semi-)automatic liquid handlers are equipped for.



- 6.1 Label a clean 50 mL (Falcon) tube with the sample identification.
Pour the entire volume of a Tempus tube (approx. 9 mL) into a fresh 50 mL (Falcon) tube
- 6.2 Use 1X PBS (phosphate-buffered saline) to a **total volume of 12 mL**.

Note

If the initial blood sample was less than 3 mL, make up the difference by adding more PBS.

- 6.3 **Vortex** for 30-45 sec at highest power.
Make sure that the tube is securely capped. Frothing of the sample after vortexing is normal.

- 6.4 Centrifuge samples  4000 x g, 4°C, 01:00:00 , (+medium brake)

1h

- 6.5 Carefully pour the supernatant from each tube into a **new** 50 mL tube (*keep new tube for later gDNA isolation*). Do so in **one movement**, in order to not dislodge the RNA pellet.

Note

The pellet will be at the bottom wall of the tube and might appear as pink-ish or white-ish smudge. Sometimes the pellet can be barely visible.

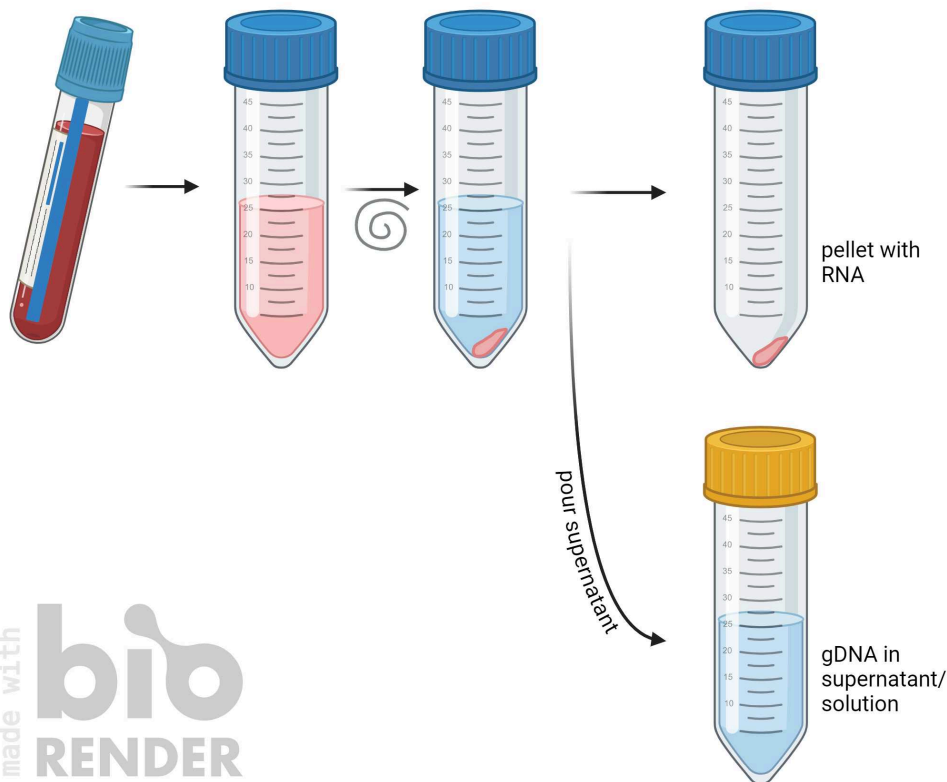
Use a paper towel to take off drops from the rim of the RNA 50 mL tube.

Starting the next step with a minimal amount of supernatant is essential, therefore try to drain as much buffer as possible without over-drying or disturbing the pellet.

Note

We keep the supernatant at 4°C if gDNA isolation proceeds within 24h, or store it at -20°C for several weeks, if the gDNA isolation cannot be done the following day.

Pellet can be air-dried (lid off), if you feel uncomfortable using paper towels.



Simplified workflow; lid colors of tubes are only exemplary. Figure © ZeBanC, created using biorender.com

- 6.6 Add 300 µL Homogenisation (+THG) buffer (at RT) to every tube and resuspend pellet by vortexing (15sec).

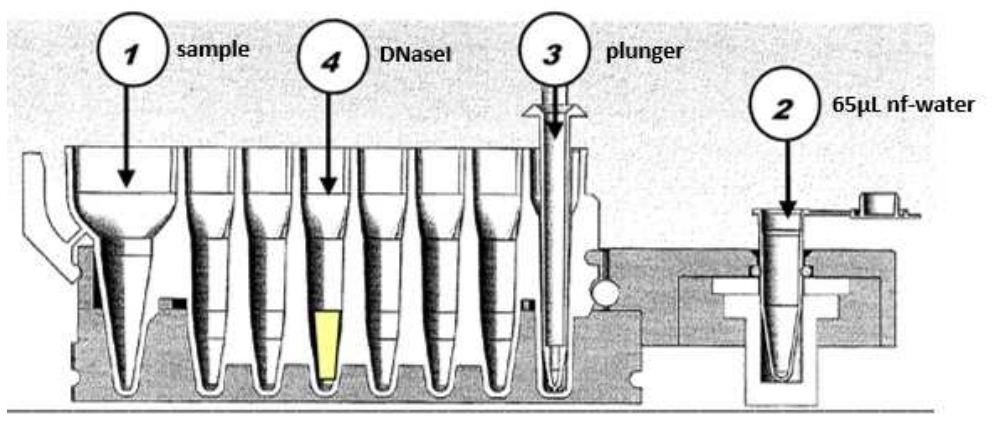
6.7 Add 200 μ L Lysis Buffer (Promega #MC501C) and 25 μ L Proteinase K (PK) Solution (Promega #MC500C) per sample, vortex very shortly (approx 1 sec) and incubate at RT for 10min.

6.8 Transfer complete lysate into the first well of the **Maxwell Cartridge** (Promega #AS138A). Load the rest of the cartridge according to the Maxwell® RSC simplyRNA Blood Kit (#AS1380) instructions.

Add 10 μ L DNase-I solution to well number 4 (shown in yellow).

Place a 0.5 ml tube (part of Promega Kit #AS1380) in the corresponding slot on the Maxwell® RSC tray (see figure, position 2). Label this tube according to your samples.

Add 65 μ L nuclease-free water into the 0,5 ml tube.



Cartridge loading scheme. Figure © Promega

6.9 Load and execute the protocol for "simplyRNA Blood" on the Maxwell® RSC instrument. Running time is approx. 45min.



Equipment

Maxwell RSC instrument

NAME

Automated nucleic acid purification platform

TYPE

Promega

BRAND

AS4500

SKU

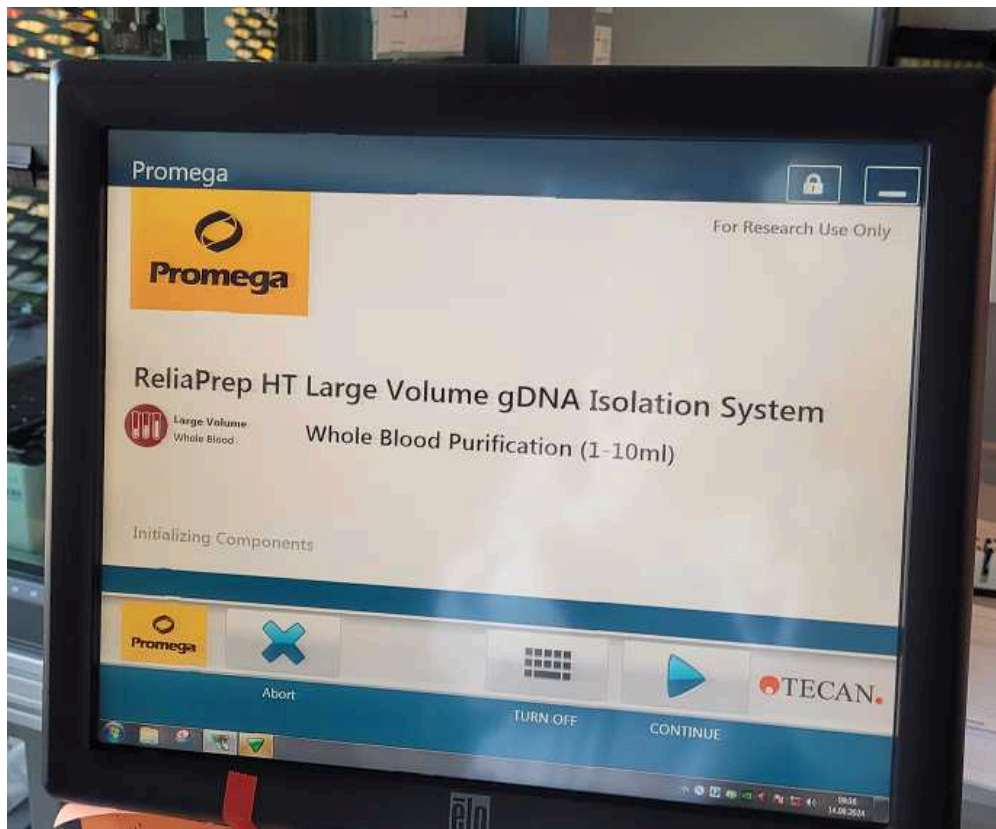
Note

Our final RNA volume is approx 40uL and concentrations rank between 20-500 ng/uL, with an average of 130 ng/uL. *In our experience*, initial blood draw, underfilling and improper mixing of blood with the Tempus stabilizer have the biggest impact on nucleic acid yield

gDNA Isolation from supernatant

5h

- 7 On a **TECAN Evo Freedom Instrument**, run the following protocol on 8–10 ml of the **supernatants from step 6.5**. This protocol is **fully automated** (steps 7.2–7.13) and normally does not require hands-on interventions.
Other automated systems (i.e. Hamilton Microlab STAR, PerkinElmer JANUS etc) should be able to run the same or a similar protocol.



on Tecan, load the ReliaPrep HT Large Volume gDNA Isolation System protocol. Picture © ZeBanC



ReliaPrep™ Large Volume HT gDNA Isolation System. Picture © Promega

ReliaPrep™ Large Volume HT gDNA Isolation System **Promega Catalog #A2751**

Equipment

Freedom EVO-2 HSM2 (custom)	NAME
liquid handling and robotics workstation	TYPE
TECAN (RRID:SCR_016771)	BRAND
30084252	SKU
https://lifesciences.tecan.com/freedom-evo-platform	LINK

Note

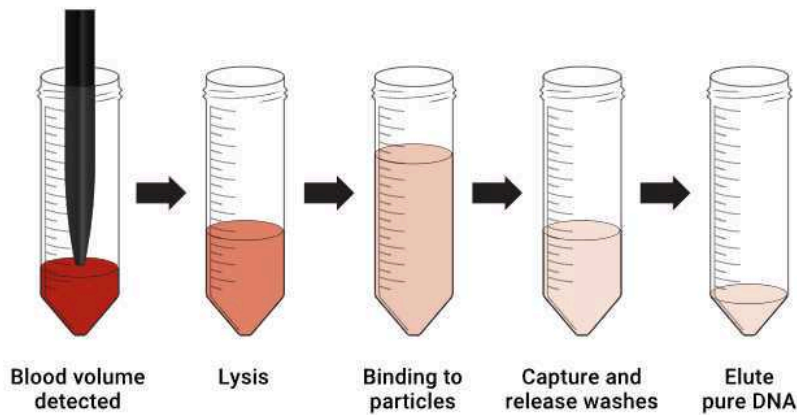
Thaw supernatants (if frozen) at RT or at 4°C over night.
Load the 50 mL (Falcon) tubes with the supernatant into the instrument.

7.1 Load the instrument with **bulk reagents** (see table).

Alkaline Protease	45
ReliaPrepResin	35
NFW	27
20xTE	5
Proteinase K	9
Lysisbuffer	620
Binding Buffer	680
Prepared Wash Buffer	810
50% EtOH	590

Usual bulk load (all in mL) for one run of 32 samples

7.2 In a first step the actual volume of the sample is measured in order to calculate the volumes of subsequent reagents.



Working principle of the ReliaPrep™ Large Volume HT gDNA Isolation System. *Figure © Promega*

- 7.3 **Proteinase K Working Solution (0.02volumes;** 60–90µL, based on the starting sample volume) is added to each tube and shaken at 500rpm, 25°C for 1 min.
- 7.4 **Alkaline Protease (0.125volumes;** 375–600µL) is added to each sample and shaken at 500rpm, 25°C for 1 min.
- 7.5 **1volume** (3–5mL) of **Lysis Buffer** is added to each sample.
- 7.6 After Lysis Buffer is added, the samples are **incubated** at 65°C for 30 minutes while shaking at 500rpm, followed by 10 minutes of shaking at 500rpm at 25°C.
- 7.7 Next, **1.2volumes** (3600 – 5600µL) of **Binding Buffer** is added to each sample and shaken at 500rpm, 25°C for 3 min.
- 7.8 **ReliaPrep™ Resin** is thoroughly mixed and **0.1volumes** (300–500µL) of resin is added to each sample. Binding of gDNA to the resin happens during incubation at 25°C for 20 minutes at 550rpm followed by magnetic capture for 20 minutes to collect the resin.
- 7.9 Waste from the lysis and binding is removed (by vacuum pump), then 5–7ml of **Wash Buffer** is added to each tube. The magnet then releases the resin into the Wash Buffer.
- 7.10 Samples are shaken at 500rpm, 25°C, for 2 minutes. Afterwards they are tip-mixed to thoroughly disperse the resin. Following this, the instrument mixes by shaking at 500rpm for 2 more minutes.



- 7.11 Waste from the first wash is removed from each tube, and 4-9ml of **Wash Buffer** is added (based on the original sample volume) while shaking at 500rpm for 4 more minutes.
- 7.12 Next, the resin is magnetically captured for 3 minutes, the waste removed and the beads are resuspended in 4-6mL of **50% Ethanol for a last wash step**. Then the instrument then shakes at 500rpm for 4 minutes and subsequently captures the resin magnetically for 3 minutes.
- 7.13 All waste is removed and **525uL low-TE Buffer** is added to each tube. Samples are shaken at 500rpm for 3 minutes, then at 400rpm for 15 minutes at 80°C. Magnetic capture is performed for 4 minutes to deplete sample of the resin, and the eluates are transferred to 2D-barcoded tubes (SBS rack).

Note

SBS racks (for barcoded tubes) have the same layout as a 96-well PCR plate; which could also be used.

- 7.14 The instrument is finished.
Nucleic acid concentration is determined by either **NanoDrop or Quantus measurement**.

Note

Usual volume of gDNA is approx. 450-400ul and concentrations range between 50-150ng/uL, with an average of 100ng/uL.



Protocol references

This SOP, as used in the Central Biobank Charité, was developed with the help of and using individual Kits manufactured by Promega (RRID:SCR_006724).

<https://www.promega.de/products/nucleic-acid-extraction/rna/maxwell-rsc-simplyrna-blood-kit/?catNum=AS1380>

<https://www.promega.de/products/nucleic-acid-extraction/genomic-dna/reliaprep-large-volume-ht-gdna-isolation-system/?catNum=A2751>

We are aware, that other methods (for gDNA isolation from Tempus tubes) have also been successfully established and do NOT claim novelty of this approach.

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