



May 05, 2026

Phenol-free RNA extraction of *Rubus armeniacus* berries

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvwwr4dvmk/v1>

Katharina Wolff^{1,1}, Boas Pucker^{1,1}

¹Plant Biotechnology and Bioinformatics, IZMB, University of Bonn



Katharina Wolff

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.e6nvwwr4dvmk/v1>

Protocol Citation: Katharina Wolff, Boas Pucker 2026. Phenol-free RNA extraction of *Rubus armeniacus* berries. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.e6nvwwr4dvmk/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We have used this protocols successfully for *Rubus armeniacus* berries

Created: April 30, 2026



Last Modified: May 05, 2026

Protocol Integer ID: 316038

Keywords: RNA extraction, berry, free rna extraction of rubus armeniacus, berries of rubus armeniacus, extraction of rna, free rna extraction, berry, rna, rubus armeniacus, extraction, phenol

Abstract

Protocol optimized for the extraction of RNA out of berries of *Rubus armeniacus*.

Materials

Materials

Materials: mortar & pestle, water bath, centrifuge for 2 ml tubes with cooling function, NanoDrop

RNA Extraction Buffer

[M] 300 millimolar (mM) TRIS HCL (pH 8.0)

[M] 25 millimolar (mM) EDTA (pH 8.0)

[M] 2 Molarity (M) NaCl

[M] 2 Mass / % volume CTAB

[M] 2 Mass / % volume PVPP

[M] 2 % (v/v) β -mercaptoethanol

β -mercaptoethanol is added directly prior to extraction

TE Buffer

[M] 10 millimolar (mM) TRIS (pH 8.0)

[M] 0.1 millimolar (mM) EDTA (pH 8.0)

This Buffer needs to be RNase free

Choloroform:Isoamylalkohol (24:1)

10 M LiCl

Ethanol (abs.)

70% Ethanol (ice cold)

N2



Safety warnings

- ⚠ β -ME is a neurotoxin. Use gloves when handling it.
Perform all chloroform procedures under the fume hood.

Before start

This protocol is optimized for the extraction of RNA from berries of *Rubus armeniacus* and has been successfully applied to berry tissue from different ripening stages



day 1

- 1 For each berry prepare 2 × 2mL reaction tube with 2% β-mercaptoethanol in  900 μL of Extraction Buffer (make sure the buffer has been mixed as PVPP is not soluble) and preheat at  65 °C
- 2 Homogenize 1 frozen berry with mortar and pestle in liquid N₂
- 3 Add homogenized material (powder) to the two tubes of preheated extraction buffer and resuspend carefully
(It is crucial to completely resuspend the powder. Avoid transfer of ice.)
- 4 Incubate at  65 °C for  00:20:00 and invert tubes frequently to carefully mix the solution.
- 5 Centrifuge the samples at  11000 x g, 4°C, 00:10:00
- 6 Transfer the supernatant to fresh 2 mL reaction tubes
- 7 Add an  equal volume of Chloroform:Isoamylalcohol (24:1) to the tubes and centrifuge at  12000 x g, 4°C, 00:10:00
- 8 Transfer upper phase into a fresh tube and repeat the extraction with an  equal volume of Chloroform:Isoamylalcohol (24:1) and centrifuge  12000 x g, 4°C, 00:10:00
- 9 Transfer the upper phase to a clean 1.5 mL reaction tube and add  1/4 volume of 10 M LiCl and incubate the sample overnight at  4 °C

day 2



- 10 Centrifuge the samples  12000 x g, 4°C, 00:20:00
- 11 Discard supernatant and wash pellet with  500 µL 70% ice cold ethanol
- 12 Centrifuge  12000 x g, 4°C, 00:05:00
- 13 Decant ethanol and dissolve RNA pellet in  100 µL TE buffer
- 14 Add  300 µL ice cold ethanol (abs) and gently mix by inversion
- 15 Centrifuge at  12000 x g, 4°C, 00:05:00
- 16 Discard supernatant and dissolve RNA pellet in  20 µL of TE buffer
- 17 Perform quality checks of RNA such as NanoDrop measurements and Gel electrophoresis and store at  -80 °C

Protocol references

This RNA extraction has been based on the methods of :

Wang, K., Liu, M., Cui, F., Asiegbu, F., 2021. A simple phenol-free isolation method for high-quality RNA from bilberry. *MethodsX* 8, 101481. <https://doi.org/10.1016/j.mex.2021.101481>

Reid, K.E., Olsson, N., Schlosser, J., Peng, F., Lund, S.T., 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. *BMC Plant Biol.* 6, 27. <https://doi.org/10.1186/1471-2229-6-27>