Preparation of lysis buffers for the extraction of viral RNA for the detection of a SARS-CoV-2-infection

Paul Klemm[1], Linda Lattermann[3], Blerina Shkodra-Pula[1], Karoline F. Haupt[5], David Pretzel[1], Christine Weber[1], Stephanie Schubert[2,4], Anja Traeger[1,2], Uwe Köhn[1], Stephan König[3], Bettina Löffler[5], Michael Baier[5], Stefanie Deinhardt-Emmer[5]*, Ulrich S. Schubert[1,2]*

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[1]Chair of Organic Chemistry and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Humboldtstrasse 10, 07743 Jena. www.schubert-group.de; E-mail: ulrich.schubert@uni-jena.de

[2]Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Philosophenweg 7, 07743 Jena.

[3]TrophoSYS GmbH, Botzstrasse 5, 07743 Jena.

[4]Institute for Pharmaceutical Technology and Biopharmacy, Friedrich Schiller University Jena, Lessingstrasse 8, 07743 Jena.

[5]Institute for Medical Microbiology, University Hospital Jena, Am Klinikum 1, 07747 Jena. E-mail: stefanie.deinhardt-emmer@med.uni-jena.de

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Preamble: The quantitative real-time polymerase chain reaction (q-RT-PCR) is essential for the identification of patients with coronavirus (SARS-CoV-2 / Covid-19). Prior to q-RT-PCR, the viral RNA must be isolated from the samples. Due to supply bottlenecks of commercially available RNA isolation kits and their components, six easily prepared buffer solutions were tested for their potential as cell lysis- and extraction buffers. In the following, the recipes of the individual buffer solutions as well as the results of ribonucleic acid extraction from respiratory material of positively tested SARS-CoV-2 patients are listed. All buffers were implicated in the standard protocol of the QIAamp viral RNA Mini Kit from Qiagen (Hilden, Germany) and tested using the purification columns from the RNeasy Mini Kit from Qiagen. The usage of the

RNeasy columns and the washing buffers AW1 and AW2 from the QIAamp DNA Mini Kit resulted in a comparable detection (RIDAGene, R-Biopharm) of the E gene in the q-RT-PCR. The buffer solutions 1 and 2 are based on the denaturing and chaotrophic substance guanidinium thiocyanate. Both buffer solutions are suitable for the isolation of intact viral RNA and can be used as an alternative to commercial buffer solutions of unknown composition (Table 3). Due to the toxicity of 2-mercaptoethanol, buffer solution 2 is preferable to buffer solution 1, as it prevents exposure to the performer with this substance. Guanidinium thiocyanate-free buffer solutions 3 and 4 can also be used to isolate genetic material, but with lower efficiency (Table 3). If the potent guanidinium salt is not available, these buffer solutions can be used. Buffer solutions 5 and 6 do not show successful isolation of viral RNA (Table 3). Thus, an alternative to the guanidinium thiocyanate-based commercial buffers could be shown.

A) Buffer solutions with high potential

Buffer solution 1 (basis: chaotropic substance guanidinium thiocyanate): Preparation protocol for 120 mL

56.717 g (480 mmol) guanidinium thiocyanate were dissolved in an Erlenmeyer flask in 60 mL ultrapure water under the influence of ultrasound at 65 °C. In a separate Erlenmeyer flask, 0.882 g (3 mmol) sodium citrate dihydrate were dissolved in 10 mL ultrapure water under constant stirring. The sodium citrate solution obtained was adjusted to pH = 7.0 using 0.1 molar hydrochloric acid and added to the guanidinium thiocyanate solution. Then, 0.601 g (2.05 mmol) sarcosyl (sodium salt) and 0.837 mL (12 mmol) 2-mercaptoethanol (fume hood!) were added and shaken until a clear solution was obtained. This solution was filled up to 120 mL with ultrapure water.

Due to the existing toxicity of 2-mercaptoethanol, according to the GESTIS substance database on 2-mercaptoethanol: 244 mg/kg (LD_{50}, rat, oral) and 167 mg/kg (LD_{50}, rabbit, transdermal), the usage of protective clothing (lab coat, gloves and safety glasses) and working under a fume hood is required. In the literature, an expiry date of one month is given for the buffer when stored at room temperature.

For the preparation of larger buffer quantities, it is recommended to prepare a stock solution of the aqueous sodium citrate solution (pH = 7.0) as well as a higher concentrated hydrochloric acid. The preset sodium citrate solution can be used for dissolving guanidinium thiocyanate in addition to ultrapure water.

Table 1: Final concentration of the individual components, amounts for preparation of 10 L buffer, and suppliers.

	Final concentration	Mass	Volume	Supplier	Order number
Guanidinium thiocyanate	4 mol/L	4726.4 g		Carl Roth	2628.1
Sodium citrate dihydrate	0.025 mol/L	73.525 g		Carl Roth	4088.1
alternative: NaCitrat (1 M) (pH = 7.0)	0.025 mol/L		250 mL		
Na Sarcosyl	0.01 mol/L	29.338 g		Sigma Aldrich	L9150
2-Mercaptoethanol	0.1 mol/L	78.13 g	69.76 mL	Sigma Aldrich	63689

Reference: P. Chomczynski, N. Sacchi, "The Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate–Phenol–Chloroform Extraction: Twenty-Something Years On", *Nature Protocols* **2006**, *1*, 581-585.

Buffer solution 2 (basis: chaotropic substance guanidinium thiocyanate): Preparation protocol for 204 mL

In an Erlenmeyer flask, 1.576 g (10 mmol) Tris-Cl was dissolved in 60 mL ultrapure water with constant stirring. The pH value was adjusted to 6.4 using 0.1 molar sodium hydroxide solution, and the solution was filled up to a volume of 100 mL using ultrapure water. 120 g (1016 mmol) guanidinium thiocyanate were weighed directly into the storage vessel and dissolved by complete addition of the previously prepared Tris-Cl solution using ultrasound at 65 °C. In a separate Erlenmeyer flask, 18.612 g (50 mmol) Na_2EDTA dihydrate (disodium salt) were mixed with 80 mL ultrapure water and dissolved by adding sodium hydroxide pellets while stirring continuously. The EDTA solution obtained was adjusted to pH = 8.0 using 0.1 molar sodium hydroxide solution, filtered and filled up to 100 mL with ultrapure water. 8.8 mL of this EDTA solution were added to the guanidinium thiocyanate solution. Afterwards, 2.455 mL (4.2 mmol) Triton X-100 were added and shaken until a homogeneous solution was available.

For the preparation of larger buffer amounts it is recommended to prepare stock solutions of EDTA and Tris-Cl as well as a higher concentrated sodium hydroxide solution.

Table 2: Final concentration of the individual components, amounts for preparation of 10 L buffer, and suppliers.

	Final concentration	Mass	Volume	Supplier	Order number
Guanidinium thiocyanate	5 mol/L	5908 g		Carl Roth	2628.1
Tris-Cl (0.1 M, pH = 6.4)	0.05 mol/L		5 L	Carl Roth	9090.1
EDTA (0.5 M, pH = 8.0)	0.022 mol/L		440 mL	Carl Roth	8043.1
Triton X-100	1.2 wt/v	120 g	112.15 mL	Sigma Aldrich	T8787

Reference: R. Boom, C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen, J. van der Noordaa, "Rapid and Simple Method for Purification of Nucleic Acids", *Journal of Clinical Microbiology* **1990**, *28*, 495-503.

The results of the q-RT-PCR of all tested buffer solutions are summarized in Table 3.

Table 3: Overview of the q-RT-PCR results of the tested buffers.

#	Buffer solution	Pat. sample	Cq value potential buffer	Cq value Qiagen
1	Guanidinium Lysis Buffer 1	vi6460	26.01	26.32
2	Guanidinium Lysis Buffer 2	vi6446	25.66	27.85
3	Triton X	vi6460	30.04	26.32
4	SDS Lysis Buffer I (+ fresh DTT)	vi6446	32.33	27.85
5	SDS Lysis Buffer II	vi6460	/	26.32
6	NP-40 Lysis Buffer	vi6446	/	27.85

Table 4: Overview of the q-RT-PCR results of the tested buffers with the comparison of the Qiagen viral RNA column and the RNeasy Mini Kit column.

#	Buffer solution	Cq value (viral RNA Kit)	Cq value (RNeasy Mini Kit)
1	Guanidinium Lysis Buffer 1	16.56	16.59
2	Guanidinium Lysis Buffer 2	15.59	15.97

B) Buffer solutions with moderate potential (alternative to guanidinium thiocyanate)

Buffer solution 3 (Triton X): Preparation protocol for 120 mL

1.050 g (18 mmol) sodium chloride and 0.189 g (1.2 mmol) Tris-Cl were dissolved in 80 mL ultrapure water with constant stirring. To the resulting solution 0.3 mL (0.51 mmol), Triton X-100 was added and homogenized using ultrasound. The pH value of the solution was adjusted to 7.4 using a 0.1 molar sodium hydroxide solution. The volume of the solution was filled up to 120 mL with ultrapure water.

Table 5: Final concentration of the individual components, amounts for preparation of 10 L buffer, and suppliers.

	Final concentration	Mass	Volume	Supplier	Order number
Sodium chloride	0.15 mol/L	87.66 g		Fluka	11984051
Tris-Cl	0.01 mol/L	15.76 g		Carl Roth	9090.1
Triton X-100	0.25%	26.75 g	25 mL	Sigma Aldrich	T8787

Reference: K. Shatzkes, B. Teferedegne, H. Murata, "A Simple, Inexpensive Method for Preparing Cell Lysates Suitable for Downstream Reverse Transcription Quantitative PCR", *Scientific Reports* **2014**, *4*, 4659.

Buffer solution 4 (SDS Lysis Buffer I): Preparation protocol for 120 mL

0.600 g (2.08 mmol) sodium lauryl sulfate and 0.946 g (6 mmol) Tris-Cl were dissolved in 80 mL ultrapure water with constant stirring. The pH value of the solution was adjusted to 7.4 with a 0.1 molar sodium hydroxide solution. The volume of the solution was filled up to 120 mL with ultrapure water. 1.2 g (7.78 mmol) dithiothreitol were added to the solution immediately before using the buffer and shaken until a clear solution was obtained.

Table 6: Final concentration of the individual components, amounts for preparation of 10 L buffer, and suppliers.

	Final concentration	Mass	Supplier	Order number
Sodium lauryl sulfate	0.5 wt%	50 g	Carl Roth	L9150
Tris-Cl	0.05 mol/L	78.8 g	Carl Roth	9090.1
Dithiothreitol	1 wt%	100 g	Fisher Sci.	R0862

Reference: https://en.wikipedia.org/wiki/Lysis_buffer, 20.03.2020, 15:00.

C) Buffer solutions without potential

Buffer solution 5 (SDS Lysis Buffer II): Preparation protocol for 100 mL

2 g (6.93 mmol) sodium lauryl sulfate, 0.584 g (10 mmol) sodium chloride and 6.057 g (50 mmol) Tris were dissolved in 80 mL ultrapure water while stirring continuously. The pH value of the solution was adjusted to 8.0 with concentrated hydrochloric acid. The volume of the solution was filled up to 100 mL with ultrapure water. Contrary to the original instructions, 8 mg/mL milk powder /g sediment were not added to the addition.

Table 7: Final concentration of the individual components, amounts for preparation of 10 L buffer, and suppliers.

	Final concentration	Mass	Supplier	Order number
Sodium lauryl sulfate	2 wt%	200 g	Carl Roth	L9150
Tris	0.5 mol/L	605.7 g	Carl Roth	0188.1
Sodium chloride	0.1 mol/L	1000 g	Fluka	11984051

Reference: T. Miura, Y. Masago, D. Sano, T. Omura, "Development of an Effective Method for Recovery of Viral Genomic RNA from Environmental Silty Sediments for Quantitative Molecular Detection", *Applied and Environmental Microbiology* **2011**, *77*, 3975.

Buffer solution 6 (NP-40 Lysis Buffer): Preparation protocol for 120 mL

1.050 g (18 mmol) sodium chloride and 0.945 g (6 mmol) Tris-Cl were dissolved in 80 mL ultrapure water while stirring continuously. To the resulting solution, 1.2 mL (2.05 mmol) Triton X-100 was added and homogenized using ultrasound. The pH value of the solution was adjusted to 7.4 with a 0.1 molar sodium hydroxide solution. The volume of the solution was filled up to 120 mL with ultrapure water.

Table 8: Final concentration of the individual components, amounts for preparation of 10 L buffer, and suppliers.

	Final concentration	Amount	Volume	Supplier	Order number
Sodium chloride	0.15 mol/L	87.66 g		Fluka	11984051
Tris-Cl	0.05 mol/L	78.8 g		Carl Roth	9090.1
Triton X-100	1%	107 g	100 mL	Sigma Aldrich	T8787

Reference: https://en.wikipedia.org/wiki/Lysis_buffer, 20.03.2020, 15:00.

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