

DETAILED DESCRIPTION OF THE INVENTION

~~Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.~~

10 In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be
15 understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise. For example, if in a preferred embodiment the chaotropic agent of the lysis buffer
20 of the invention is guanidinium hydrochloride and in another preferred embodiment the reducing agent of the lysis buffer is dithiothreitol, it is a preferred embodiment of the present invention that guanidinium hydrochloride and dithiothreitol are present in the lysis buffer according to the present invention.

25 Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", H.G.W. Leuenberger, B. Nagel, and H. Kölbl, Eds., Helvetica Chimica Acta, CH-4010 Basel, Switzerland, (1995).

30 The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, and recombinant DNA techniques which are explained in the literature in the field (cf., e.g., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

fourth aspect of the present invention, the method according to the fifth aspect of the present invention, or the method according to the sixth aspect of the present invention.

The present invention provides for the first time a lysis buffer, a use, a processing method, and a kit which are universally applicable to the lysis of bodily samples. This allows for the first time to perform the lysis of several types of different bodily samples, such as bodily samples relevant for the diagnosis of a respiratory disease, in parallel using the same lysis buffer and processing protocol, and thus, to process different types of bodily samples in a high-throughput setting. Furthermore, the present invention provides a valuable alternative to existing approaches for the processing, preferably the lysis of bodily samples, in particular such bodily samples which are difficult to handle, e.g., respiratory samples such as sputum or tracheal secretions, especially if isolation of nucleic acids and subsequent analysis thereof is required. Some of the advantages of the present invention are a low infection risk, especially if highly pathogenic organisms are to be detected, efficient solubilization/liquefaction and/or lysis of highly viscous samples, reduced handling, possibility for automating the process and performing the process in a high-throughput setting even for different sample types, and compatibility to different DNA isolation methods.

EXAMPLES

The following examples serve to illustrate particularly preferred embodiments of the present invention and are not to be construed as limiting the scope of the invention in any manner. Those skilled in the art will recognize that various modifications may be made to the foregoing description and the following examples without departing from the spirit and scope of the invention.

Example 1: Process Details and Overview

Materials and Equipment

consumables and chemicals		
item	supplier	ordering number
screw cap tubes (1.5 ml)	e.g., neolab	1-6186
screw caps	e.g., neolab	1-6193
glass beads, 425 - 600 μ m, acid-washed	Sigma-Aldrich	G8772
lysis buffer AL	Qiagen	19075

CLAIMS

1. Use of ~~Aa~~ lysis buffer comprising:
 - (i) at least one chaotropic agent,
 - (ii) at least one reducing agent, and
 - (iii) at least one proteolytic enzymefor the lysis of highly viscous bodily samples.
2. The ~~use lysis buffer~~ according to claim 1, ~~wherein the lysis buffer further comprises~~ing beads, preferably made of a solid inert material such as glass, ceramics, plastics, or metal such as steel.
3. The ~~use lysis buffer~~ according to claim 1 or 2, ~~which wherein the lysis buffer~~ is universally applicable for the lysis of highly viscous bodily samples that are relevant for the diagnosis of a respiratory disease.
4. The ~~use lysis buffer~~ according to any one of claims 1 to 3, wherein the at least one chaotropic agent is selected from the group consisting of guanidinium thiocyanate, guanidinium isothiocyanate, guanidinium hydrochloride, guanidinium chloride, alkali thiocyanate, alkali isothiocyanate, alkali iodide, and alkali perchlorate, and/or the at least one reducing agent is selected from the group consisting of dithiothreitol (DTT), N-acetyl-cysteine (NALC), beta-mercaptoethanol, Tris(2-carboxyethyl) phosphine (TCEP), and thioredoxin, and/or the at least one proteolytic enzyme is selected from the group consisting of a serine protease and a cysteine protease, preferably the at least one proteolytic enzyme is selected from the group consisting of proteinase K, elastase, subtilisin, and caspase.
5. The ~~use lysis buffer~~ according to any one of claims 1 to 4, ~~which wherein the lysis buffer~~ does not contain any other enzyme than the at least one proteolytic enzyme.
6. ~~Use of:~~
 - (i) ~~at least one chaotropic agent,~~
 - (ii) ~~at least one reducing agent, and~~
 - (iii) ~~at least one proteolytic enzyme~~

for the lysis of a broad spectrum of bodily samples.

- 5 | 67. A method for processing a highly viscous bodily sample comprising the step of (i) providing a mixture comprising the highly viscous bodily sample to be processed, at least one chaotropic agent, at least one reducing agent, and at least one proteolytic enzyme, preferably further comprising the step of (ii) heating the mixture to a first temperature, and/or preferably further comprising the step of (iii) heating the mixture to a second temperature, and/or preferably further comprising the step of (iv) bead milling the mixture.
- 10 | 78. The method according to claim 67, wherein step (iv) is performed simultaneously to or during step (iii).
- | 89. The method according to claim 67 or 78, wherein steps (ii) to (iv) are performed in an automated process.
- 15 | 940. The method according to any one of claims 67 to 89, further comprising the step of (v) isolating a nucleic acid from the mixture, preferably using a silica-based or magnetic bead-based nucleic acid isolation technology.
- 20 | 1044. A method for analyzing a highly viscous bodily sample comprising processing the highly viscous bodily sample according to the method of any one of claims 67 to 940 and (vi) applying the mixture to a nucleic acid amplification/analysis method.
- 25 | 1142. A method for detecting the presence of a pathogen in a highly viscous bodily sample comprising processing the highly viscous bodily sample according to the method of any one of claims 67 to 940 and (vi) applying the mixture to a nucleic acid amplification/analysis method that is suitable for detection of said pathogen, wherein preferably the pathogen is associated with a respiratory disease.
- 30 | 1243. A method for diagnosing a respiratory disease in a subject comprising processing a highly viscous bodily sample according to the method of any one of claims 67 to 940 and (vi) applying the mixture to a method that is suitable for diagnosing the respiratory disease,

wherein preferably the respiratory disease is an infectious respiratory disease or a respiratory tumor.

- 5 | 1314. A method for processing at least two highly viscous bodily samples comprising processing each of the at least two highly viscous bodily samples according to the method of any one of claims 67 to 910, wherein the at least two highly viscous bodily samples are different types of highly viscous bodily samples.
- 10 | 1415. A lysate of a highly viscous bodily sample comprising a bodily sample, at least one chaotropic agent, at least one reducing agent, and at least one proteolytic enzyme.
- 15 | ~~16. A lysate obtainable by processing a bodily sample according to the method of any one of claims 7 to 10.~~
- ~~17. A kit comprising:~~
- ~~(i) a chaotropic agent,~~
 - ~~(ii) a reducing agent,~~
 - ~~(iii) a proteolytic enzyme, and~~
 - ~~(iv) an instruction leaflet for processing a bodily sample.~~