Instructions for use
InviPrep® Fast Lysis Buffer

INVITEK diagnostics

Language: EN

REF 1001136100

100 preparations

Invitek Molecular GmbH
Robert-Rössle-Straße 10
13125 Berlin
Germany

Important notes

Thank you for purchasing the **InviPrep® Fast Lysis Buffer** from Invitek Diagnostics.

The product serves the purpose of manual isolation of DNA from bacteria and fungi in culture broth samples (e.g. pre-enriched food samples) or culture plate samples.

Due to the fast lysis technology DNA is isolated in less than 20 minutes. Extracts can be directly used in downstream applications, such as PCR.

WARNING! Improper handling and use for other than the intended purpose can cause danger and damage. Therefore, we ask you to read through these instructions for use and follow them carefully. Always keep them handy. To avoid personal injury, also observe the safety instructions.

All versions of the instructions for use can be found on our website for download or can be requested from us: www.invitek.com

Contact:

Technical support:

techsupport@invitek.com

GERMANY

Robert-Rössle-Str. 10, 13125 Berlin, Germany

+ 49 30 9489 2908

PORTUGAL

Zona Industrial de Tondela, ZIM II, Lote 6, 3460-070 Tondela, Portugal

+351 232 817 817

Table of Contents

1. Safety instructions

Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- When and while working with chemicals, always wear protective clothing, disposable gloves and safety glasses.
- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- Do not reuse any consumables.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar airflow until the samples are lysed.

Before handling chemicals read and understand all applicable safety data sheets (MSDS). These are available online at www.invitek.com.

Dispose of kit residues and waste fluids in accordance with your country's regulations, again refer to the MSDS. Invitek Molecular has not tested the liquid waste generated by the kit for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely but cannot be excluded completely. Therefore, liquid waste must be considered infectious and must be handled and disposed of according to local safety regulations.

European Community risk and safety phrases for the components of the **InviPrep® Fast Lysis Buffer** to which they apply are listed below as follows:

For the **InviPrep® Fast Lysis Buffer** no risk and safety phases apply.

2. Product information

2.1 Kit contents

	100 purifications
Catalogue No.	1001136100
Fast Lysis Buffer	20 ml/bottle
Short Protocol	1 leaflet

2.2 Reagents and equipment to be supplied by user

Lab equipment:

- Microcentrifuge
- Thermo shaker (99°C)
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer
- Safe-Lock Reaction tubes (1.5 ml, or 2.0 ml)

Liquids and solvents:

- DNase/RNase free water

2.3 Storage, appearance and shelf life

Shelf life: InviPrep® Fast Lysis Buffer should be stored at room temperature and has a shelf life as indicated on the outer kit package label.

Cloudy precipitates may form in the buffer, these do not affect the function of the buffer. **Shake the buffer well before each use** to ensure even homogenisation of buffer components.

After opening, InviPrep® Fast Lysis Buffer has a shelf life of 3 months.

Room temperature (RT) is defined as a range from 15-30°C.

2.4 Intended use

The **InviPrep® Fast Lysis Buffer** is intended for the manual isolation and purification of DNA from bacteria and fungi in culture broth (e.g., pre-enriched food samples) or culture plate samples. The buffer is suitable for DNA isolation from gram-positive and gram-negative bacteria, including difficult-to-lyse bacteria like *Listeria monocytogenes* and other foodborne pathogens. It can also be used for DNA isolation from a wide range of different fungi (yeast and mold) including Aspergillus sp., Candida sp., Geotrichum sp., Penicillium sp. and Saccharomyces sp..

The **InviPrep® Fast Lysis Buffer** was successfully tested with a wide range different pre-enriched food samples like meat, dairy, egg, sea food and fatty samples like cheese.

Due to the fast lysis technology DNA is isolated in less than 20 minutes. Extracts can be directly used in downstream applications, such as PCR.

The **InviPrep® Fast Lysis Buffer** contains no harmful chemicals and works without any time-consuming spin column purification.

The product is not intended to be used in diagnostic procedures.

2.5 Product information and specifications

Starting material	Yield	Quality	Time
Up to 2 ml liquid samples from culture broth samples (e.g., pre-enriched food samples such as meat, dairy, egg or sea food) or other liquids Colonies from culture plates	Depending on sample type (storage and source)	Depending on sample type (storage and source)	approx. 20 min for 24 samples

Yield and quality of purified nucleic acids depend on the sample type, sample source, transport, storage, and age.

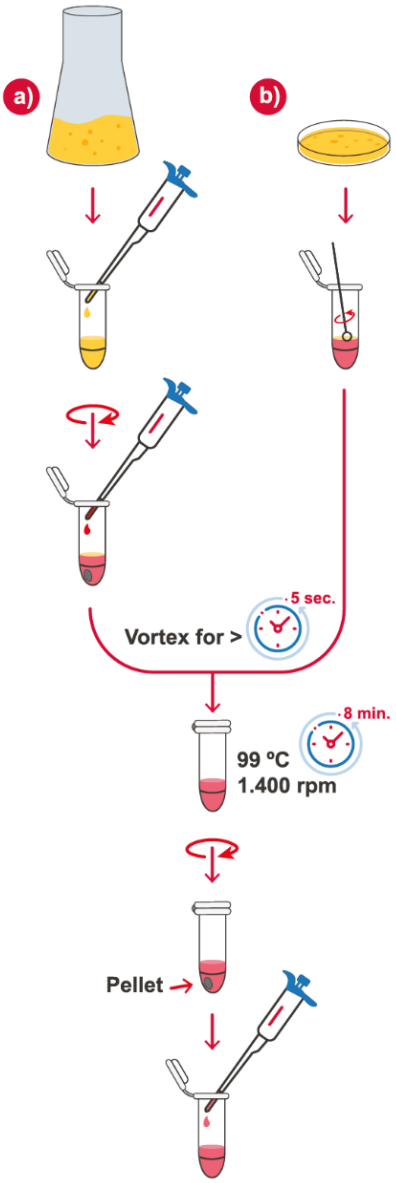
Downstream Applications:
Yield and quality of isolated nucleic acids are suitable for plenty of molecular-analytic applications via amplification methods such as PCR or LAMP techniques, including next-generation sequencing (NGS)-based 16S and ITS rRNA Sequencing. Downstream applications should be performed according to the respective manufacturers' specifications.

2.6 Principle and procedure

The InviPrep® Fast Lysis Buffer causes a fast, direct lysis of gram-positive and gram-negative bacteria as well as fungi (yeast and mold) without the use of toxic chemicals. DNA is released and potential inhibitors are removed from the eluate. Thus, the DNA can be used directly for downstream molecular biology applications such as PCR or LAMP analysis.

3. Extraction of DNA from bacteria and fungi with the InviPrep® Fast Lysis Buffer

3.1 Short Protocol InviPrep® Fast Lysis Buffer



1. Shake the buffer well before use.

2. Preheat thermo shaker to 99°C.

3. **a) DNA Purification from liquid culture (e.g. culture broth)**
   Transfer up to 2 ml liquid sample to a Safe-Lock tube and centrifuge at max. speed (>10.000 x g) for 2 min to pellet cells.
   Carefully remove supernatant, do not disturb the pellet!

   Add **200 µl** of **Fast Lysis Buffer** to the tube.

   b) DNA purification from culture plates
   Pick a colony or collect a small loopful of cells from a culture plate.
   Transfer cells to **200 µl** of **Fast Lysis Buffer** in a Safe-Lock tube.

   Note: *For processing difficult samples please refer to the instructions for use, chapters 3.2 and 3.3.*

4. Resuspend by vortexing for at least 5 sec.

5. Incubate sample on thermo shaker for 8 min at 99°C while continuously shaking (e.g. 1.400 rpm).

   Note: *Ensure that Safe-Lock tubes are tightly closed!*

6. Cool down sample tube for 2 min at RT.

7. Centrifuge at max. speed (>10.000 x g) for 2 min.

8. Transfer ~50 µl of the supernatant to a fresh tube for subsequent analysis.
   The supernatant can be stored at 4 °C up to 1 week or at −20 °C for longer periods.

   Note: *Dilute supernatant 1:5 in DNase/RNase free water for bacterial cultures and 1:100 for fungal cultures before downstream analysis.*

3.2 Protocol 1: DNA purification from liquid samples (e.g., culture broth samples such as pre-enriched food samples)

Shake the buffer well before use!

1. Preheat thermo shaker to 99°C.
2. Transfer up to 2 ml liquid sample to a Safe-Lock tube and centrifuge at max. speed (>10.000 x g) for 2 min to pellet cells.

   Note: Samples with many food residues should be pre-cleared prior to this step by an additional centrifugation at 900 x g for 2 min. Transfer the supernatant into a fresh tube and proceed as described in step 2.
3. Carefully remove supernatant. Do not disturb the pellet.

   Note: *For samples showing a significant fat layer after the first centrifugation step, carefully remove fat together with supernatant. Add the 200 µl Fast Lysis Buffer, resuspend using a pipette and transfer mixture to a fresh tube. Continue with step 5.*
4. Add **200 µl** of **Fast Lysis Buffer** to the tube.
5. Resuspend by vortexing for at least 5 sec.
6. Incubate sample on thermo shaker for 8 min at 99°C while continuously shaking (e.g. 1.400 rpm).

   Note: *Ensure that Safe-Lock tubes are tightly closed!*
7. Cool down sample tube for 2 min at RT.
8. Centrifuge at max. speed (>10.000 x g) for 2 min.
9. Transfer ~50 µl of the supernatant to a fresh tube for subsequent analysis. The supernatant can be stored at 4 °C up to 1 week or at −20 °C for longer periods.

   Note: *In case of PCR inhibition, eluates should be diluted 1:5 in DNase/RNase free water for downstream analysis.*

3.3 Protocol 2: DNA purification from culture plates

Shake the buffer well before use!

1. Preheat thermo shaker to 99°C.
2. Pick a colony or collect a small loopful of cells from a culture plate.

   Note: *Avoid collecting spores when using spore-building fungi. Avoid taking agar.*
3. Transfer cells to **200 µl** of **Fast Lysis Buffer** in a Safe-Lock tube.
4. Resuspend by vortexing for at least 5 sec.
5. Incubate sample on thermo shaker for 8 min at 99°C while continuously shaking (e.g. 1.400 rpm).

   Note: *Ensure that Safe-Lock tubes are tightly closed!*
6. Cool down sample tube for 2 min at RT.
7. Centrifuge at max. speed (>10.000 x g) for 2 min.
8. For bacterial cultures: transfer 50 µl of the supernatant to a fresh tube containing 200 µl of DNase/ RNase free water and mix well for subsequent analysis.

   For fungal cultures: transfer 10 µl of the supernatant to a fresh tube containing 990 µl of DNase/ RNase free water and mix well for subsequent analysis.

   The supernatant can be stored at 4 °C up to 1 week or at -20 °C for longer periods.

   Note: *If required, the amount of water can be adjusted to achieve a higher or lower dilution of the eluate (see section 4.1 Troubleshooting).*

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Low amount of nucleic acids	Bacterial pellet lost	Avoid disturbing the pellet when removing the supernatant.
	Incorrect storage of starting material	Ensure that starting material is appropriately stored. Avoid repeated thaw-freeze cycles of the sample material.
	Low nucleic acid concentration in the sample	Try using a higher amount of starting material or try to use undiluted eluates for downstream analysis.
Degraded nucleic acids	Incorrect storage of starting material	Ensure the sample is taken and stored correctly, please refer to the FAQ section on our webpage for more information.
	Old material	Ensure the sample is taken and stored correctly, please refer to the FAQ section on our webpage for more information.
Nucleic acids do not perform well in downstream applications (e.g., PCR)	Inhibition	Dilute eluate in PCR grade water (e.g., 1:5, 1:10, 1:100 or 1:1000). Avoid collecting spores when using spore-building fungi.
	Carryover of sample pellet	When transferring the eluate to a fresh tube, strictly avoid transferring any pelleted material from the bottom of the tube.
	Low nucleic acid concentration in the eluate	Try to use less diluted or undiluted eluate for downstream analysis.

4.2 Warranty

Invitek Molecular guarantees the correct function of the kit for applications described in this manual and in accordance with the intended use. In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all kit components has been tested to ensure product quality.
Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection. Immediately upon receipt, inspect the product to ensure that it is complete and intact. In the event of any discrepancies, you must inform Invitek Molecular immediately in writing. Modifications of the kit and protocols and use that deviate from the intended purpose are not covered by any warranty.

Invitek Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
Invitek Molecular warrants products as set forth in the General Terms and Conditions available at www.invitek.com. If you have any questions, please contact techsupport@invitek.com.

4.3 Symbols used on product and labeling

 Manufacturer

 Lot number

 Unique device identifier

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Amount of sample preparations

4.4 Further documents and supplementary information

Visit www.invitek.com for further information on:

- FAQs and troubleshooting tips
- Manuals in different languages
- Safety data Sheets (MSDS)
- Web support
- Product videos

If, despite careful study of the operating instructions and further information, you still require assistance, please contact us at techsupport@invitek.com or the dealer responsible for you.

4.5 Ordering information

Product	Package Size	Catalogue No.
InviPrep® Fast Lysis Buffer	100 preparations	1001136100

Revision history

Revision	Date	Description
EN-v1-2023	2023-07-14	New document
EN-v2-2024	2024-02-20	Protocol for DNA extraction from fungi.

invitek.com

INVITEK
diagnostics

PORTUGAL
Zona Industrial de Tondela, ZIM II, Lote 2 e 6
63460-070 Tondela
Portugal

Phone: +351 232 817 817

GERMANY
Robert-Rössle-Str. 10
13125 Berlin
Germany

Phone: +49 30 9489 2908

info@invitek.com
www.invitek.com

2024-02-20 EN-v2-2024