Instructions for use
Invisorb® Spin Plasmid Miniprep Kit

INVITEK diagnostics

InviSorb®

Language: EN

RUO

REF 1010110300

Σ 50 preparations
250 preparations

ALS Life Sciences Portugal, S.A.
Zona Industrial de Tondela, ZIM II,
Lote 6, 3460-070 Tondela
Portugal

Important notes

Thank you for purchasing the **InviSorb® Spin Plasmid Miniprep Kit** from Invitek Diagnostics.

The **InviSorb® Spin Plasmid Miniprep Kit** is for the fast and efficient purification of high-copy plasmid DNA from 0.5 ml to 2 ml bacterial suspension, using Spin Column technology.

WARNING! Improper handling and use for other than the intended purpose can cause danger and damage. Therefore, we ask you to read 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

Technical support:

techsupport@invitek.com

GERMANY

Haynauer Str. 60, 12249 Berlin, Germany

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 kit residues and waste fluids in accordance with your country's regulations, again refer to the MSDS. Invitek Diagnostics has not evaluated 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 **InviSorb® Spin Plasmid Miniprep Kit** to which they apply are listed below as follows:

Solution B

Warning

Hazard statements
H315 - Causes skin irritation.
H319 - Causes serious eye irritation.
Precautionary statements
P264 - Wash hands, forearms and face thoroughly after handling.
P280 - Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P337+P313 - If eye irritation persists: Get medical advice/attention.

Solution C

Warning

Contains: guanidinium chloride; guanadine hydrochloride
Hazard statements
H302 - Harmful if swallowed.
H315 - Causes skin irritation.
H319 - Causes serious eye irritation.
Precautionary statements
P264 - Wash hands, forearms and face thoroughly after handling.
P280 - Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P337+P313 - If eye irritation persists: Get medical advice/attention.

RNase Solution

Danger

Hazard Statements
H334- May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary Statements
P261- Avoid breathing mist or vapors.
P284- Wear respiratory protection.
P501- Dispose of contents/ container to an approved waste disposal plant.

Emergency medical information can be obtained 24 hours a day from infotrac, www.infotrac.net:

outside of USA: **1 – 352 – 323 – 3500**
in USA: **1 – 800 – 535 – 5053**

2. Product information

2.1 Kit contents

	250 purifications
Catalogue No.	1010110300
Solution A	70 ml/bottle
Solution B	70 ml/bottle
Solution C	70 ml/bottle
RNase Solution	700 µl/vial
Wash Buffer	60 ml/bottle (final volume 200 ml)
Elution Buffer	60 ml/bottle
RTA Spin Filter Set	250 pieces
1.5 ml Receiver Tubes	250 pieces
Short Protocol	1 leaflet

2.2 Reagents and equipment to be supplied by user

Lab equipment:

- Microcentrifuge
- Thermo mixer
- Measuring cylinders
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer

Liquids and solvents:

- 96 - 100 % ethanol (non-denatured)
- Optional: Lysozyme (10 mg/ml)

2.3 Storage, appearance, and shelf life

Shelf life: All buffers and kit components should be stored at room temperature and have a shelf life as indicated on the outer kit package label.

After opening, individual components of the kit, as well as components prepared accordingly before first use, have a shelf life of 3 months.

Before each use, make sure that all components are at room temperature. If there are temperature-related precipitates in the solutions, dissolve them by carefully warming (up to 30°C).

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

Wash Buffer: after adding ethanol, it should be firmly closed and stored at room temperature.

2.4 Intended use

The **InviSorb® Spin Plasmid Miniprep Kit** is a Spin Column based nucleic acid extraction kit, intended for the manual isolation and purification of plasmid DNA. The kit can be used for overnight bacterial culture of 0.5 ml to 2 ml sample volume or for bacterial pellets.

The product is intended for use by professionals only, such as laboratory technicians, physicians and biologists trained in molecular biological techniques and *in vitro* diagnostic procedures.

2.5 Product information and specifications

Starting material	Yield	Quality	Time
0.5 - 2.0 ml bacteria suspension Bacterial pellets from max. 2 ml* suspension	Up to 20 µg	A_{260} : A_{280} 1.8 – 2.1	< 15 min

*Standard application, low copy plasmids may require higher volumes, refer to chapter 3.5

The yield obtained depends on the bacterial culture and plasmid purified.
The **InviSorb® Spin Plasmid Miniprep Kit** is suitable for the purification of plasmid vectors and derivates such as pBR322, pUC, pBluescript and others. The kit can be used for the purification of plasmids of bacteria grown in common culture media like e.g. Lambda broth, LB medium, SOC medium or tryptone broth.

Downstream Applications:
Yield and quality of isolated nucleic acids are in general suitable for plenty of molecular applications such as PCR techniques, Restriction Enzyme Digestion, Cloning, Sequencing and *In-vitro* translation. Downstream applications should be performed according to the respective manufacturer's specifications.

2.6 Principle and procedure

1. Lyse samples
Bacterial cells are harvested and resuspended in Solution A. RNase A is added to digest RNA during sample lysis which is initialized by adding Solution B. Solution B contains SDS which solubilizes proteins and phospholipids in the bacterial cell membrane. Subsequently the lysate is neutralized by adding Solution C. Proteins are denatured; chromosomal DNA, cellular debris, and SDS are precipitated, while the smaller plasmid DNA renatures correctly and stays in the solution.

2. Bind plasmid DNA
After a centrifugation step, the supernatant of each lysate is applied to a RTA Spin filter. Plasmid DNA is adsorbed to the membrane of the column, while digested RNA and cellular proteins pass the column.

3. Wash to remove residual contaminations
Contaminations like endonucleases are washed away using Wash Buffer, while the plasmid DNA remains bound to the membrane.

4. Elute plasmid DNA
Plasmid DNA is eluted from the RTA Spin Filter using 50 - 100 µl Elution Buffer.

3. Nucleic acid extraction with the InviSorb® Spin Plasmid Miniprep Kit

3.1 Before starting a protocol

When using the kit for the first time make sure all buffers and reagents are prepared as indicated:

Buffer and reagent preparations prior first use: 250 purifications
Wash Buffer: Add 140 ml of **96 - 100% ethanol** to the bottle. Mix thoroughly, always keep the bottles firmly closed.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps should be carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not mix kit components with components from other kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

3.2 Growth of bacterial cultures

Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 ml medium containing the appropriate selective antibiotic. Incubate for 12–16 h at 37°C with vigorous shaking.

Growth for more than 16 h is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

Harvest the bacterial cells by centrifugation at minimum 6.000 x g in a table-top microcentrifuge for 3 min at room temperature. The bacterial cells can also be harvested in 15 ml centrifuge tubes at 5.400 x g for 10 min at 4°C. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

Important note:

Incomplete removal of the bacteria culture medium will affect lysis and dilute the lysate.

3.3 Short protocol InviSorb® Spin Plasmid Miniprep Kit

Lyse samples

Refer to chapter 3.2 “Growth of bacterial cultures” for plasmid specific treatment.

1. Transfer 0.5 – 2 ml bacterial culture into a 1.5 ml or 2.0 ml microcentrifuge tube (not provided).
2. Centrifuge for 1 min at max. speed.
3. Remove supernatant completely.
4. Resuspend bacterial pellet in 250 µl **Solution A**, vortex vigorously.
5. Add 2.5 µl **RNase Solution**.
6. Add 250 µl **Solution B**, mix gently by inverting the tube 4-6 times.
7. Add 250 µl **Solution C**, mix gently by inverting the tube 4-6 times.
8. Centrifuge for 5 min at max. speed.

Bind nucleic acids

9. Transfer clarified supernatant into the RTA Spin Filter Set.
10. Incubate for 1 min at RT.
11. Centrifuge for 1 min at 11.000 x g.

Wash to remove residual contaminations

12. Discard filtrate.
13. Add 750 µl **Wash Buffer.**
14. Centrifuge for 1 min at 11.000 x g.
15. Discard filtrate.

Elute nucleic acids

16. Centrifuge for 3 min at 11.000 x g.
17. Place Spin Filter into a new 1.5 ml Receiver Tube.
18. Add 50 – 100 µl **Elution Buffer.**
19. Incubate for 1 min at RT.
20. Centrifuge at 11.000 x g for 1 min to elute plasmid DNA.

3.4 Protocol 1: Preparation of plasmid DNA from 0.5 – 2.0 ml bacteria cultures

Note: When referring to max. speed for the centrifuge make sure it has a minimum speed of 17.000 x g.

1. Transfer 0.5 ml to 2 ml of the bacterial culture into a 1.5 ml or 2.0 ml microcentrifuge tube.

   For larger volumes of starting material please check protocol 2 and 3.
2. Centrifuge for 1 min at max. speed to pellet the cells.
3. Remove the supernatant as completely as possible. Try not to aspirate any remaining cells.
4. Resuspend the cell pellet in 250 µl **Solution A** completely by vortexing or by pipetting up and down. For multiple processing of samples, we recommend using a vortexer. ***Note****: No cell pellet or clumps should be visible!*
5. Add 2.5 µl of liquid **RNase Solution** (alternatively you make a Master Mix with **Solution A** beforehand).
6. Add 250 µl **Solution B**, close the tube and mix carefully by inverting the tube 4-6 times. Do not perform the lysis step more than 5 min! *Do not vortex!*

   Important: *Do not vortex the tube to mix the suspension! This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. This sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA.*
7. Add 250 µl **Solution C** and mix gently, but thoroughly, by inverting the tube 4-6 times*. Do not vortex!*
8. Centrifuge for 5 min at max. speed.
   During centrifugation time, label the needed amount of RTA Spin Filter Sets.
9. Transfer the clarified supernatant into the RTA Spin Filter Set. *Avoid disturbing the pellet!*
10. Incubate for 1 min on to the RTA Spin Filter Set.
11. Centrifuge for 1 min at 11.000 x g.
12. Discard the filtrate.
13. Add 750 µl **Wash Buffer**.
14. Centrifuge for 1 min at 11.000 x g.
15. Discard the filtrate.
16. Centrifuge for 3 min at max. speed to complete removing the residual ethanol.
17. Place the Spin Filter into a new 1.5 ml Receiver Tube.
18. Add 50 - 100 µl **Elution Buffer** directly onto the center of the Spin Filter surface.
19. Incubate for 1 min at room temperature.
20. Centrifuge at 11.000 x g for 1 min to elute the plasmid DNA.

Note*: To increase the final DNA yield we recommend using a higher volume of* ***Elution Buffer****. A longer incubation time with Elution Buffer (up to 10 min) also leads to a slightly higher final yield. In order to increase the DNA concentration, we recommend eluting in a smaller volume than 50 µl (minimal 30 µl), but this will lead to a reduction of yield.*

Note*: For in vitro transcription application, please elute the plasmid DNA with ddH_2O.*

3.5 Protocol 2: Purification of low-copy plasmids & cosmids from up to 10 ml bacteria culture

Transfer 1 ml to 10 ml of the bacterial culture into a 15 ml Falcon Tube and spin down the bacterial cells.

Follow the protocol 1, but: it is recommended to double the volumes of **Solution A, B, C**.

When plasmids or cosmids are bigger than 10 kb, prewarm **Elution Buffer** to 70°C prior eluting DNA from the Spin Filter membrane.

A 10 ml overnight culture typically yields 5–10 µg DNA.

***Note**: This is an additional application, and the needed buffer volumes are not calculated in the provided buffers.*

3.6 Protocol 3: Purification of plasmid DNA from 2 ml gram-positive bacteria cultures

Note: When referring to max. speed for the centrifuge make sure it has a minimum speed of 17.000 x g.

1. Transfer 0.5 ml to 2 ml of the bacterial culture into a 1.5 ml or 2.0 ml microcentrifuge tube.
2. Centrifuge for 1 min at maximum speed to pellet the cells; remove the supernatant as completely as possible.
3. Resuspend the cell pellet in 250 µl **Solution A** completely by vortexing or by pipetting up and down. Add 2.5 µl of **RNase Solution** and 10 µl of lysozyme (10mg/ml or according to the producers' instructions, not provided). Mix the suspension.
4. Incubate for 10 min at 37°C.

Follow protocol 1 starting at step 6.

4. Appendix

4.1 Troubleshooting

Problem	Possible Cause	Recommendation
Low yield of plasmid DNA	Incorrect Wash Buffer	Prepare the Wash Buffer exactly as described in the manual, including adding ethanol. Ensure the Wash Buffer's cap is firmly fixed for storage.
	Poor elution of plasmid DNA	Add the Elution Buffer directly onto the center of the Spin Filter.
	Conditions for bacterial cultures are not optimal	Adjust the conditions (media, growing time, etc.) as necessary.
	Elution Buffer incorrect	Use only low-salt buffers (e.g., Elution Buffer or water) for elution, ensuring pH is between 7.0 – 8.0. If using water, verify the pH is within this range.
	Too much starting material	Use only the recommended amount of bacterial suspension.
Contamination of plasmid DNA with chromosomal DNA	Sample was mixed too vigorously	Follow the protocol precisely; do not vortex. Mix samples by inverting the tubes carefully.
	Bacteria overgrown	Reduce the time for growing the bacterial culture.
	Lysis too long	Ensure lysis does not exceed 5 minutes.
Problems due to poor cleavage by restriction endonucleases or other applications	Contamination of the final plasmid DNA with salt components	Wash the plasmid DNA bound on the Spin Filters as described.
	Contamination of the final DNA with ethanol	Keep the given centrifugation time, extend it if necessary to ensure ethanol is fully removed (test by smell).
RNA contamination	RNase digestion insufficient	Check culture volume against recommended volumes and reduce if necessary. Add more RNase A. Recover DNA by precipitating the eluate, digesting with RNase A, and purifying on a new spin column.
Additional band below the super coiled plasmid DNA band	Denatured super coiled plasmid DNA	Incorrect incubation in Solution B can cause denaturation. Increase incubation time with Solution B cautiously to avoid denaturing the super coiled plasmid DNA.

4.2 Warranty

Invitek Diagnostics 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 and ISO 9001 certified Quality Management System the performance of all kit components has been assessed to ensure product quality.
Any problems, incidents or defects shall be reported to Invitek Diagnostics 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 Diagnostics immediately in writing. Modifications of the kit and protocols and use that deviate from the intended purpose are not covered by any warranty.

Invitek Diagnostics reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
Invitek Diagnostics 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 labelling

 Manufacturer

 Lot number

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Amount of sample preparations

 Research Use Only

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.
InviSorb® Spin Plasmid Miniprep Kit	250 preparations	1010110300

Revision history

Revision	Date	Description
DE 582.01	2025-07-31	New document

INVITEK
diagnostics

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

Phone: +351 232 817 817

GERMANY
Haynauer Str. 60, 12249
Berlin, Germany

info@invitek.com
www.invitek.com