For food testing purposes

FOR *IN VITRO* USE ONLY

foodproof® StarPrep One Kit

Version 5, January 2017

For rapid preparation of PCR templates from bacterial enrichment cultures.

Order No. S 400 07

21 ml

Order No. S 400 07 L

105 ml

Store the kit at 15 to 25 °C

Table of contents

1. What this Product Does

The **food**proof StarPrep One Kit is designed for the rapid preparation of DNA from Gram-negative bacteria like *Salmonella* or *Cronobacter* for direct use in PCR. Up to 96 samples can be processed in parallel. In less than 30 minutes, preparation with this Lysis Buffer yield PCR template DNA from 100 µl (or more) of enrichment cultures. The obtained DNA can be used directly in any PCR application. The special Lysis Buffer eliminates the need for hazardous organic extractions or chaotropic agents. The reduced number of handling steps results in time savings and, because transfer steps of DNA containing extracts are not necessary, cross-contamination risks are minimized as well as the exposure to biohazardous material.

Kit Contents

Product	Content	Storage
S 400 07	3 bottles with 7 ml Lysis Buffer and a magnetic stir bar	15 to 25 °C
S400 07 L	5 bottles with 21 ml Lysis Buffer and a magnetic stir bar	15 to 25 °C

Storage and Stability

The components of the **food**proof® StarPrep One Kit are guaranteed to be stable through the expiration date printed on the label when stored at 15 to 25 °C.

Additional Equipment Required – Procedure A for low throughput

- Standard tabletop microcentrifuge capable of a 13,000 × g centrifugal force
- Heating Unit
- Orbital Shaker (Vortex)
- Optional: Magnetic Stirrer

Additional Equipment Required – Procedure B for high throughput

	Order No.	Description
Heating Block	D 110 38	TH 21 Heating Block Thermostat
	D 110 39	Exchange Block for DWP for TH 21
Consumables	Z 100 72	96er MicroTube System: 8-Strip Tubes, 1.2 ml
	Z 100 73	8-Cap Strips

- Centrifuge with swing-out rotor for microtiter plates capable of a 5,800 × g centrifugal force, (*e.g.* Rotanta 460, Order No. D 110 34 - D 110 37)

- Multichannel pipette and deep well tips, (*e.g.* EP Xplorer Plus Electronic Multichannel Pipette, 50 – 1,250 µl, Order No. D 110 40/ Z 100 58)
- Sterile reservoir (Order No. Z 100 60)

Additional Equipment Required – Procedure C for ultra-rapid high throughput

	Order No.	Description
Heating Block	D 110 38	TH 21 Heating Block Thermostat
	D 110 39	Exchange Block for DWP for TH 21
Consumables	Z 100 72	96er MicroTube System: 8-Strip Tubes, 1.2 ml
	Z 100 73	8-Cap Strips

- Microcentrifuge capable of a 2000 × g centrifugal force
- Multichannel pipette and deep well tips, (*e.g.* EP Xplorer Plus Electronic Multichannel Pipette, 50 – 1,250 µl, Order No. D 110 40/ Z 100 58)
- Sterile reservoir (Order No. Z 100 60)

All additionally required equipment for Procedures B and C can be ordered at BIOTECON Diagnostics.

Applicability Statement

The Lysis Buffer can be used to prepare DNA from 100 µl (or more) of Gram-negative bacterial enrichment cultures.

The Lysis Buffer is optimized for the preparation of enrichment cultures of various types of sample material. The quality of the DNA obtained with the Lysis Buffer is suitable for any PCR application. The **food**proof StarPrep One Kit is MicroVal, NordVal International and AOAC-RI validated for a variety of foods.

Isolation Procedure

In order to avoid cross-contamination use filter tips. Follow all universal safety precautions governing work with biohazardous materials (*e.g.,* wear lab coats and gloves at all times). Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

2. How to Use this Product

To avoid foam formation of the Lysis Buffer, do not shake the bottles up and down. Mix thoroughly while pipetting the buffer for sample preparation. For mixing, use a magnetic stirrer at low speed to move the stir bar in the bottle (high speed might result in topple over of the bottle). Alternatively, shake the bottle before every pipetting step by moving it horizontally on the lab bench.

Procedure A for low throughput

Before you Begin

Warm the heating unit to 95 – 100 °C.
The following protocol describes the DNA isolation from 100 µl enrichment culture.

Step	Action	Volume	Time/g Time/Temp.
1	Shake the enrichment culture gently and let settle.		5 – 10 min
2	Transfer the sample (supernatant) to a 1.5 ml reaction tube. **Note:** For very cloudy supernatants, a reduction of the sample volume (*e.g.* 50 µl) might enhance the DNA isolation efficiency.	100 µl	
3	Centrifuge. **Note:** If the enrichment cultures are totally clear, centrifugation at ≥ 13,000 × g is recommended. Use *e.g.* latex beads* to increase efficiency and yield a visible pellet.		5 min at 8,000 × g
4	Remove the supernatant with a pipette immediately after centrifugation, discard, and inactivate appropriately. **Note:** Take care that the tip of the pipette in the reaction tube is on the opposite side of the pellet.		
5	Add Lysis Buffer. **Note:** Use a magnetic stirrer (low speed) or shake the bottle with Lysis Buffer gently in a short time interval to avoid sedimentation of ingredients.	200 µl	
6	Resuspend the pellet by vortexing or by pipetting gently up and down. **Note:** For optimal DNA isolation efficiency the pellet has to be completely resuspended.		
7	Incubate the suspension in a heating unit.		10 min at 95 – 100 °C
8	Carefully remove the reaction tube from the heating unit, allow the tube to sit. **Note:** As the tube will be hot, use forceps for removal.		1 min at 15 – 25 °C
9	Mix by vortexing.		2 s
10	Centrifuge. Result: The supernatant now contains the extracted DNA and can be used directly for PCR. **Note:** Parts of the sediment may inhibit PCR and must not be used for PCR.		2 min at 13,000 × g

*Sigma Catalog-No. LB-11; add 10 µl of the suspension (1:10 in distilled water).

Note: If necessary, the centrifugation forces should be calculated according to the manual of the used centrifuge.

Alternative Procedures

The following protocol describes the DNA isolation from samples with a high amount of the target organism (*e.g.,* pure cultures or colonies from agar plates).

A. For liquid enrichment cultures

Step	Action	Volume	Time/g Time/Temp.
1	Shake the enrichment culture gently and let settle.		5 – 10 min
2	Pipet Lysis Buffer in a new 1.5 ml reaction tube. **Note**: Use a magnetic stirrer (low speed) or shake the bottle with Lysis Buffer gently in a short time interval to avoid sedimentation of ingredients.	200 µl	
3	Transfer the sample (supernatant) to the 1.5 ml reaction tube containing the Lysis Buffer.	50 µl	
4	Mix by vortexing or pipetting gently up and down, and continue with step 7 of the above procedure.		

B. For bacterial colonies

Step	Action	Volume	Time/g Time/Temp.
1	Pipet Lysis Buffer in a new 1.5 ml reaction tube. **Note**: Use a magnetic stirrer (low speed) or shake the bottle with Lysis Buffer gently in a short time interval to avoid sedimentation of ingredients.	200 µl	
2	Transfer a small part of the colony with a suitable tool (*e. g.* inoculating needle) to the 1.5 ml reaction tube containing the Lysis Buffer.		
3	Mix by vortexing or pipetting gently up and down, and continue with step 7 of the above procedure.		

Procedure B for high throughput

Before you Begin

Warm the heating block TH 21 to 110 °C.

Note: The temperature of the corresponding heating unit has to be set to 110 °C to reach the required temperature of 100 °C inside the tubes.

The following protocol describes the DNA isolation from 100 µl (or more) enrichment culture using 8-strip tubes and multichannel pipettes. Two different extraction methods should be used depending on which kind of PCR-Kit (liquid or lyophilized reagents) is used for downstream processing.

A) For extracts, that will be used in combination with **food**proof LyoKits

Step	Action	Volume	Time/g Time/Temp.
1	Shake the enrichment culture gently and let settle.		5 – 10 min
2	Transfer the sample (supernatant) to the 8-tube strips. Seal with cap strips.	100 µl	
3	Centrifuge. **Note**: If the maximum speed of the used centrifuge is less than 5,800 x g, please contact BIOTECON Diagnostics.		10 min at 5,800 x g
4	Remove and discard the cap strips. Then remove the supernatant with a multichannel pipette immediately after centrifugation, discard and inactivate appropriately. **Note:** Take care that the tips of the pipette in the reaction tubes are not touching the pellets.		
5	Dilute the needed Lysis Buffer 2:1 with sterile H_2O (200 µl Lysis Buffer + 100 µl H_2O per sample) in a sterile reservoir. **Note:** Use a magnetic stirrer (low speed) or shake the bottle with Lysis Buffer gently in a short time interval to avoid sedimentation of ingredients.		
6	Add diluted Lysis Buffer. **Note:** Pipet the Lysis Buffer up and down in the reservoir before using to avoid sedimentation of ingredients.	300 µl	
7	Resuspend the pellets by stirring cautiously with the pipette tips. Seal the 8-tube strips with new cap strips. **Note:** For optimal DNA isolation efficiency the pellet has to be completely resuspended.		
8	Incubate the suspension in the heating unit for 8-tube strips. **Note**: Weight the caps down.		10 min at 100 °C
9	Carefully remove the tube strips from the heating unit, and allow the tube to sit. **Note**: As the tube will be hot, use forceps for removal.		1 min at 15 – 25 °C
10	Centrifuge Result: The supernatant now contains the extracted DNA and can be used directly for PCR. **Note**: Parts of the sediment may inhibit PCR and must not be used for PCR.		5 min at 5,800 x g

B) For extracts, that will be used in combination with **food**proof Kits (liquid).

Step	Action	Volume	Time/g Time/Temp.
1	Shake the enrichment culture gently and let settle.		5 – 10 min
2	Transfer the sample (supernatant) to the 8-tube strips. Seal with cap strips.	100 µl	
3	Centrifuge. **Note**: If the maximum speed of the used centrifuge is less than 5,800 x g, please contact BIOTECON Diagnostics.		10 min at 5,800 x g
4	Remove and discard the cap strips. Then remove the supernatant with a multichannel pipette immediately after centrifugation, discard and inactivate appropriately. **Note**: Take care that the tips of the pipette in the reaction tubes are not touching the pellets.		
5	Transfer the needed Lysis Puffer in a sterile reservoir. **Note:** Use a magnetic stirrer (low speed) or shake the bottle with Lysis Buffer gently in a short time interval to avoid sedimentation of ingredients.		
6	Add Lysis Buffer. **Note**: Pipet the Lysis Buffer up and down in the reservoir before using to avoid sedimentation of ingredients.	200 µl	
7	Resuspend the pellets by stirring cautiously with the pipette tips. Seal the 8-tube strips with new cap strips. **Note:** For optimal DNA isolation efficiency the pellet has to be completely resuspended.		
8	Incubate the suspension in the heating unit for 8-tube strips plates. **Note**: Weight the caps down.		10 min at 100 °C
9	Carefully remove the tube strips from the heating unit, and allow the tube to sit. **Note**: As the tube will be hot, use forceps for removal.		1 min at 15 – 25 °C
10	Centrifuge Result: The supernatant now contains the extracted DNA and can be used directly for PCR. **Note**: Parts of the sediment may inhibit PCR and must not be used for PCR.		5 min at 5,800 x g

Procedure C for ultra-rapid high throughput

Before you Begin

Warm the heating block TH 21 to 110 °C.

Note: The temperature of the corresponding heating unit has to be set to 110 °C to reach the required temperature of 100 °C inside the tubes.

Step	Action	Volume	Time/g Time/Temp.
1	Shake the enrichment culture gently and let settle.		5 – 10 min
2	Transfer 200 µl StarPrep One Lysis Buffer to each tube of the 8-strip.	200 µl	
3	Transfer 50 µl of the sample (supernatant) to the 8-strips. Seal the 8-strips with sterile cap strips.	50 µl	
4	Incubate the suspension in the TH21 heating unit for 8-tube strips. **Note**: Weight the caps down.		10 min at 100 °C
5	Carefully remove the tube strips from the heating unit, and allow the tube to sit. **Note**: As the tube will be hot, use forceps for removal.		1 min at 15 – 25 °C
6	Centrifuge Result: The supernatant now contains the extracted DNA and can be used directly for PCR. **Note**: Parts of the sediment may inhibit PCR and must not be used for PCR.		5 min at 2,000 x g

Quality Control

Buffered peptone water spiked with about 10^4 cfu/ml *Salmonella* is extracted as described above. 5 µl of the extract is analyzed using the foodproof *Salmonella* Detection Kit. As expected, the resulting amplification signal is obtained. The absence of contaminating DNA is controlled by an additional DNA preparation and a subsequent PCR test with unspiked broth as sample material. As expected, no amplification product is obtained.

Storage of Samples

If you want to…	Then…
Continue	Use the extracted DNA directly. Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.
Stop	Store the DNA at -15 to -25 °C for later analysis. Centrifuge again at 13,000 × g for 2 min after storage at -15 to -25 °C (procedure A) or at 5,000 x g for 5 min (procedure B) **Note:** The sample is not purified. Proteins, RNA, and other materials remain in the sample. Long-term storage or archival of prepared DNA samples is not recommended.

3. Troubleshooting

Problem	Possible cause	Recommendation
DNA sample inhibits PCR	Enrichment culture contains too many PCR inhibitors.	Perform a subcultivation (*e.g.,* 1:10 dilution in fresh enrichment broth) or dilute the DNA extract.
	Some of the centrifugation pellet transferred to the PCR.	Use the top of the supernatant as PCR template. Always centrifuge the DNA sample before performing PCR.
Low DNA yield	Inappropriate storage	Store at 15 – 25 °C.
	Enrichment culture contains substances that reduce the DNA extraction efficiency.	Perform a subcultivation (*e.g.,* 1:10 dilution in fresh enrichment broth) or reduce the sample volume.
	Pellet resuspension incomplete.	Prolong the resuspension time.
	Not enough target organisms in enrichment culture.	Prolong the incubation phase.
Lid of the reaction tube opens during or after heating	Reaction tube not firmly closed.	Ensure that all reaction tubes are firmly closed before heating. Use lid clips for closing the tubes (for Procedure A). Use a heating unit that enables removal of the tube without contact with the tube.

4. Warranty and Disclaimer of Liability

“Limited Warranty” and “Disclaimer of Liability.” BIOTECON Diagnostics warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

(1) The product is used according to the guidelines and instructions set forth in the product literature;

(2) BIOTECON Diagnostics does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by BIOTECON Diagnostics; defects caused by misuse or use contrary to the instructions supplied, or if the product is contaminated by improper storage or handling;

(3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;

(4) BIOTECON Diagnostics does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of BIOTECON Diagnostics;

(5) BIOTECON Diagnostics does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;

(6) BIOTECON Diagnostics reserves the right to replace or allow credit for any modules returned under this warranty.

5. Supplementary Information

5.1 Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

5.2 Trademarks

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Other brand or product names are trademarks of their respective holders.
Contact and Support

5.3 Contact and Support

If you have questions or experience problems with this or any other product of BIOTECON Diagnostics, please contact our Technical Support staff (for details see www.bc-diagnostics.com). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

6. Change Index

Version 1, October 2007:

First version of the package insert.

Version 2, October 2010:

Page 1 and 3: Information about large version of the kit with 500 reactions added.

Version 3, June 2013:

Page 1 and 3: Information about NordVal extension added.

Version 4, July 2014:

New Protocol for high throughput added.

Version 5, January 2017:

New Protocol for ultra-rapid high throughput added.

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