foodproof® StarPrep One Kit

Order No. S 400 07
Order No. S 400 07 L

Quick Guide

Version 4, April 2019

Introduction

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. In less than 30 minutes, preparation with this Lysis Buffer yield PCR template DNA from 100 µl (for clear samples larger volumes can be used) of enrichment cultures.

A. Kit Contents / Storage and Stability

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

B. 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 or shake the bottle before every pipetting step by moving it horizontally on the lab bench.

C. Before you Begin

Warm the heating unit to 95 – 100 °C.

D. Procedure

The following protocol describes the DNA isolation from 100 µl enrichment culture.

Step	Action	Settings
1	Shake the enrichment culture gently and let settle. Note: For sub-cultivation in small scale (*e.g.* 2 ml reaction tubes) only mix carefully by stirring with a pipette tip during step 2.	5 - 10 min
2	**Transfer** the **sample** (enrichment culture supernatant) to a 1.5 ml reaction tube. Note: For very cloudy samples, a reduction of the sample volume (*e.g.* 50 µl) might enhance the efficiency.	100 µl
3	**Centrifuge** Note: If the enrichment cultures are very 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. Take care that pipette tip is on the opposite side of the pellet in the reaction tube.	
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. 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, and **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).

E. Procedure

The following protocol includes Reagent D (Order No. A 500 02) for the detection of *Enterobacteriaceae, Cronobacter* and *Salmonella.*

Step	Action	Settings
1	Shake the enrichment culture gently and let settle. Note: For sub-cultivation in small scale (*e.g.* 2 ml reaction tubes) only mix carefully by stirring with a pipette tip during step 3.	5 - 10 min
2	**Add Reagent D** into an **empty 1.5 ml transparent** reaction **tube**. Proceed immediately with the following steps of the protocol. Avoid extended exposure to light.	300 µl
3	**Transfer** the **sample** (enrichment culture supernatant) into the reaction tube. **Mix thoroughly** by pipetting up and down. Note: For very cloudy samples, a reduction of the sample volume (*e.g.* 50 µl) might enhance the efficiency.	100 µl
4	**Incubate in the dark** at room temperature. Note: If a D-Light unit is available incubate in the unit.	5 min
5	**Expose to light** in the D-Light unit or to a high-power halogen light bulb. Note: Place the reaction tube approx. 20 cm from the high-power halogen light bulb on ice or in a cooling block.	5 min
6	**Centrifuge** Note: If the enrichment cultures are very 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
7	**Remove** the **supernatant** with a pipette immediately after centrifugation, discard and inactivate appropriately. Take care that pipette tip is on the opposite side of the pellet in the reaction tube.	
8	**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
9	**Resuspend** the **pellet** by vortexing or by pipetting gently up and down. For optimal DNA isolation efficiency, the pellet has to be completely resuspended.	
10	**Incubate** the suspension in a heating unit.	10 min 95 - 100 °C
11	Carefully **remove** the reaction **tube** from the heating unit, and **allow the tube to sit.** Note: As the tube will be hot, use forceps for removal.	1 min 15 – 25 °C
12	**Mix** by vortexing.	2 s
13	**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 13,000 × g

F. Alternative Procedure

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

F.1 For liquid enrichment cultures

Step	Action	Settings
1	Shake the enrichment culture gently and let settle.	5 - 10 min
2	**Add 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 standard procedure (D.).	

F.2 For bacterial colonies

Step	Action	Settings
1	**Add 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 standard procedure (D.).	

G. Storage of samples

If you want to…	Then…
Continue	Use the extracted DNA directly.
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. 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.

H. 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 **food**proof® *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.

For further information please refer to: https://www.bc-diagnostics.com/

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