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Kylt®

Kylt® Salmonella Purification HTP

High Throughput Purification Kit for
Salmonella pre-enrichment samples

www.kylt.eu

Kylt® Salmonella Purification HTP

Purification Kit for Salmonella pre-enrichment samples

Revision No.	Amendments
004	Combined manual for all versions of the Kylt® Salmonella Purification HTP Kit

A. Introduction

Kylt® Salmonella Purification HTP was developed as an automated method for the purification of DNA from Salmonella enrichment culture broths such as buffered peptone water (BPW) or supplemented Rappaport-Vassiliadis medium (RVS).

Kylt® Salmonella Purification HTP is designed and ideally suited to be used in conjunction with the Kylt® Purifier magnetic bead processing instrument. Other instruments may be used upon reasonable verification.

The kit can be used to purify DNA from enriched samples from veterinary and feed & food origin.

The kit is furthermore applicable to the DNA purification from bacterial isolates.

This kit is intended for use by trained laboratory staff according to standardized processes described in this manual.

When working with chemicals always wear protective lab coat, gloves and goggles and consider the indicated safety instructions.

Several different versions of the kit are available, see section B, select according to your requirements.

B. Product Variants

Product	Article No	Content	Description
Kylt® Salmonella Purification HTP	31433	384 Purifications	Kit without alcohol for individual sample numbers.
Kylt® Salmonella Purification HTP RTU	31574	384 Purifications	Ready-to-use Kit for individual sample numbers.
Kylt® Salmonella Purification Prefilled 96	31617	96 Purifications	Prefilled kit for the application on Kylt® Purifier 96.
Kylt® Salmonella Purification Prefilled 48	31618	48 Purifications	Prefilled kit for the application on Kylt® Purifier 48.

C. Sample Preparation

- **Recommendation:** Transfer at least 3 mL of the enrichment supernatant to a sterile screw-cap tube using sterile transfer pipettes, discarding the Stomacher bag. The tube serves as an intermediary container and also stores the sample for potential cultivation of Salmonellae until testing is complete.
- **Attention:** Mixing of the pre-enrichment after incubation by shaking or any other agitation must be avoided! Avoid transferring solid or greasy debris. An aliquot should be taken directly below the surface, avoiding floating particles.
- For use with bacterial isolates, pick a colony e.g., with a separate sterile pipet tip.

D. Additional Material

- Kylt® Purifier (magnetic bead processing instrument, Art. No. 31436) or Kylt® Purifier 48 (Art. No. 31748)
- Kylt® Purifier Spin Tips (Art. No. 31434)
- Kylt® Purifier Plates (Art. No. 31435) depending on the kit used.
- Micropipettes covering volumes of 10 µl to 1000 µl. Filtered tips are recommended.
- Electronic 8-channel 1000µl pipette with mixing function (recommended).
- 2-Propanol (for kit without alcohol, Art. No. 31433)
- Non-denatured Ethanol >95% (for kit without alcohol, Art. No. 31433)

E. Kit Components and Reagent Preparation

Kylt® Salmonella Purification HTP, Art. No. 31433:

Reagent	Content	Remark	GHS Label
Binding Reagent (concentrate)	4x 40 ml	Add 40 ml 2-Propanol and the contents of 1 vial of Magnetic Beads to each bottle before first use. Mix well by inverting. Shelf life after bead addition is 3 months.	
Magnetic Beads	4x 1,9 ml	Resuspend well before use. Add the contents of 1 vial to each bottle of Binding Reagent.	
Wash Solution I (concentrate)	4x 35 ml	Add 20 ml undenatured Ethanol (>95 %) to each bottle before use. Mix well.	
Wash Solution II (concentrate)	4x 12 ml	Add 45 ml undenatured Ethanol (>95 %) to each bottle before use. Mix well.	
Elution Buffer	1x 60 ml		

Kylt® Salmonella Purification HTP RTU, Art. No. 31574:

Reagent	Content	Remark	GHS Label
Binding Reagent	4x 80 ml	Add the contents of 1 vial of Magnetic Beads to each bottle before first use. Mix well. Shelf life after bead addition is 3 months.	
Magnetic Beads	4x 1,9 ml	Resuspend well before use. Add the contents of 1 vial to each bottle of Binding Reagent.	
Wash Solution I	4x 60 ml		
Wash Solution II	4x 60 ml		
Elution Buffer	1x 60 ml		

Kylt® Salmonella Purification Prefilled 96, Art. No. 31617:

Component	Content	Remark	GHS Label
Binding Plate	1	Contains Binding reagent. Remove sealing foil before use.	
Wash Plate I	1	Contains Wash Solution I. Remove sealing foil before use.	
Wash Plate II	1	Contains Wash Solution II. Remove sealing foil before use.	
Bead Plate	1	Contains Magnetic Beads. Remove sealing foil before use.	
Elution Plate	1	Contains Elution Buffer. Remove sealing foil before use.	

Kylt® Salmonella Purification Prefilled 48, Art. No. 31618:

Component	Content	Remark	GHS Label
Prefilled Plates	4	Each plate contains reagents for 16 purifications. Remove sealing foil before use.	

F. Protocol for Kylt® Salmonella Purification HTP (RTU) on Kylt® Purifier

1. Sample Transfer

- An aliquot of 500 µl per sample is transferred to each used well a Kylt® Purifier Plate (mark: "Bind").
- Alternatively, the aliquot can be mixed into pre-dispensed Binding Reagent.
- For isolates: Pick a colony and either smear it into an empty well or resuspend it into pre-dispensed Binding Reagent.

2. Plate Preparation

- Add 500 µl Wash Solution I to each used well of a Kylt® Purifier Plate (mark: "Wash I").
- Add 500 µl Wash Solution II to each used well of a Kylt® Purifier Plate (mark: "Wash II").
- Add 100 µl Elution Buffer to each used well of a Kylt® Purifier Plate (mark: "Elution").

3. Binding

- Resuspend Binding Reagent by inverting the bottle several times to obtain a homogenous suspension.
- Transfer the necessary volume to a pipetting reservoir.
- Using an electronic multi-channel pipette, transfer 750 µl of homogenous Binding Reagent to each well and mix contents twice by pipetting up and down.
- Alternatively, the Binding Reagent can be transferred after the samples.

4. Starting Kylt® Purifier

- Power on Kylt® Purifier and log in with your credentials.
- Choose "Run", choose the "Kylt® Salmonella" protocol.
- Follow instructions and load machine as prompted, column 1 of each plate should point to the inside of the machine.
- Start purification.

5. Unloading Kylt® Purifier

- The protocol is finished in about 25 minutes.
- The first accessible plate "Elution" contains the eluates.
- The eluates can be used directly in the Kylt® Salmonella spp. Real-Time PCR kit or the respective Kylt® kits for bacterial isolates.
- Transfer eluates to another plate for storage, if needed. Seal with foil seal.
- Unload Kylt® Purifier by turning the table with the buttons, discard plates accordingly.
- Clean surfaces and perform UV-decontamination if necessary.

G. Protocol for Kylt® Salmonella Purification HTP (RTU) on Kylt® Purifier 48

1. Sample Preparation

- An aliquot of 500 µl per sample is transferred to each used well in column 1 and/or 7 of the Kylt® Purifier Plate(s).
- Alternatively, the aliquot can be mixed into pre-dispensed Binding Reagent.
- For isolates: Pick a colony and either smear it into an empty well or resuspend it into pre-dispensed Binding Reagent.

2. Plate Preparation

- Add 500 µl Wash Solution I to each used well of the Kylt® Purifier Plate(s) in column 2 and/or 8.
- Add 500 µl Wash Solution II to each used well of the Kylt® Purifier Plate(s) in column 3 and/or 9.
- Add 100 µl Elution Buffer to each used well of the Kylt® Purifier Plate(s) in column 6 and/or 12.

3. Binding

- Resuspend Binding Reagent by inverting the bottle several times to obtain a homogenous suspension.
- Transfer the necessary volume to a pipetting reservoir.
- Using an electronic multi-channel pipette, transfer 750 µl of homogenous Binding Reagent to each used well in column 1 and/or 7 and mix contents twice by pipetting up and down.

4. Starting Kylt® Purifier 48

- Power on Kylt® Purifier 48 and log in with your credentials.
- Load tips onto at least the cones that are going to be used.
- Choose "Run", choose the "Kylt® Salmonella" protocol.
- Load the prepared Kylt® Purifier Plate(s) with the column 1 on the left side.
- Start purification.

5. Unloading Kylt® Purifier

- The protocol is finished in about 25 minutes.
- Column 6 and/or 12 of each used plate contains the eluates.
- The eluates can be used directly in the Kylt® Salmonella spp. Real-Time PCR kit or the respective Kylt® kits for bacterial isolates.
- Transfer eluates to another plate for storage, if needed. Seal with foil seal.
- Clean surfaces and perform UV-decontamination if necessary.

H. Protocol for Kylt® Salmonella Purification Prefilled 96 on Kylt® Purifier

1. Sample Transfer

- An aliquot of 500 µl per sample is transferred per well of the prefilled Binding Plate.
- For isolates: Pick a colony and resuspend it into a well of the prefilled Binding Plate.

2. Starting Kylt® Purifier

- Power on Kylt® Purifier and log in with your credentials.
- Choose "Run", choose the "Salm Pref" protocol.
- Follow instructions and load machine as prompted, column 1 of each plate should point to the inside of the machine.
- Start purification.

3. Unloading Kylt® Purifier

- The protocol is finished in about 25 minutes.
- The first accessible plate "Elution" contains the eluates.
- The eluates can be used directly in the Kylt® Salmonella spp. Real-Time PCR kit or the respective Kylt® kits for bacterial isolates.
- Transfer eluates to another plate for storage, if needed. Seal with foil seal.
- Unload Kylt® Purifier by turning the table with the buttons, discard plates accordingly.
- Clean surfaces and perform UV-decontamination if necessary.

I. Protocol for Kylt® Salmonella Purification Prefilled 48 on Kylt® Purifier 48

1. Sample Preparation

- An aliquot of 500 µl per sample is transferred to each used well in column 1 and 7 of the Kylt® Salmonella Purification Prefilled 48 plate(s). Mix by pipetting up and down.
- For isolates: Pick a colony Pick a colony and resuspend it into a well column 1 and 7 Kylt® Salmonella Purification Prefilled 48 plate(s).

2. Starting Kylt® Purifier 48

- Power on Kylt® Purifier 48 and log in with your credentials.
- Load tips onto at least the cones that are going to be used.
- Choose "Run", choose the "Kylt® Salmonella Prefilled" protocol.
- Load the Kylt® Salmonella Purification Prefilled 48 plate(s) with the column 1 on the left side.
- Start purification.

3. Unloading Kylt® Purifier

- The protocol is finished in about 25 minutes.
- Column 6 and/or 12 of each used plate contains the eluates.
- The eluates can be used directly in the Kylt® Salmonella spp. Real-Time PCR kit or the respective Kylt® kits for bacterial isolates.
- Transfer eluates to another plate for storage, if needed. Seal with foil seal.
- Clean surfaces and perform UV-decontamination if necessary.

E. Related and Accessory Products

Product	Article No	Content / Reactions	Description
Kylt® Salmonella spp. FLI-B 656	31019	100	Federally registered Real-Time PCR Detection Kit for Salmonella spp from primary production samples.
Kylt® Salmonella spp. 2.0	31302	100	MicroVal certified Real-Time PCR Detection Kit for Salmonella spp.
Kylt® RNA / DNA Purification HTP	31826	4 x 96	Magnetic bead based combined RNA and DNA purification kit for veterinary diagnostic samples. Suitable for Kylt® Purifier and Kylt® Purifier 48.
Kylt® Purifier	31436	1 unit	Purification system for magnetic bead based kits. Up to 96 samples are processed in under 30 minutes. Intended for high-throughput laboratories.
Kylt® Purifier 48	31748	1 unit	Purification system for magnetic bead based kits. Up to 48 samples are processed in under 30 minutes. Intended for low to medium throughput laboratories.
Kylt® Purifier Spin Tips	31434	5 Sets	Plate with 96 separate spin tips, used by the Kylt® Purifier to mix the well contents by stirring. Sufficient for 480 samples.
Kylt® Purifier Plates	31435	20 Plates	Plates to be used for the several reactions and reagents during automated nucleic acid purification. Sufficient for 320 to 480 samples (depending on device and protocol) .

Production:

AniCon Labor GmbH | Muehlenstr. 13 | D-49685 Hoeltinghausen | Germany | www.anicon.eu | www.kylt.eu | info@kylt.eu

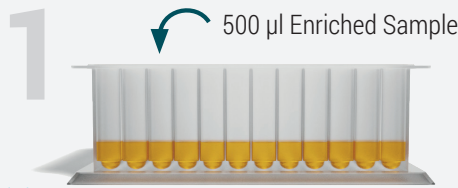
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PROTOCOL AT A GLANCE

Kylt® Salmonella Purification HTP on Kylt® Purifier



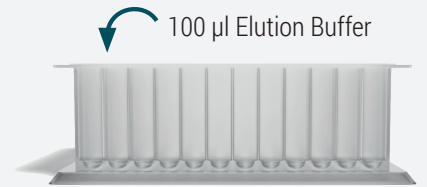
1.1 Pipet a 500 µl aliquot from each enriched sample into a well of the Kylt® Purifier Plate



2.1 Wash Plate 1



2.2 Wash Plate 2



2.3 Elution Plate



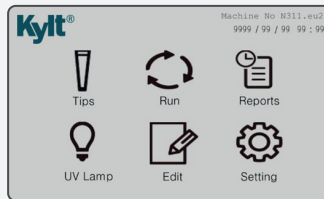
3.1 Mix Binding Reagent



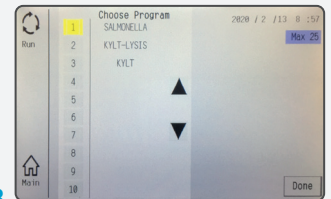
3.2 Add Binding Reagent to Binding Plate and mix contents of the wells.



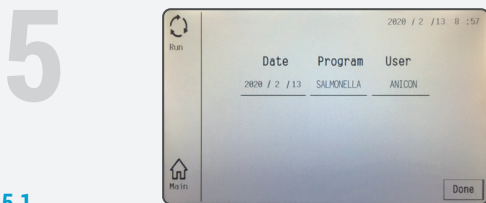
4.1 Turn on Kylt® Purifier



4.2 Click Run



4.3 Choose Kylt Salmonella program



5.1 Confirm



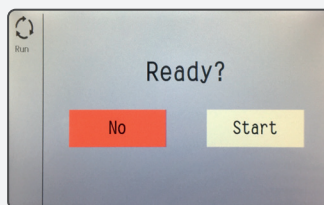
5.2 Enter Batch No, if needed



5.3 Enter sample data, if needed



6.1 Load Kylt® Purifier as prompted



6.2 When all plates are properly loaded, start process



7.1 Once protocol is finished, unload Kylt® Purifier.

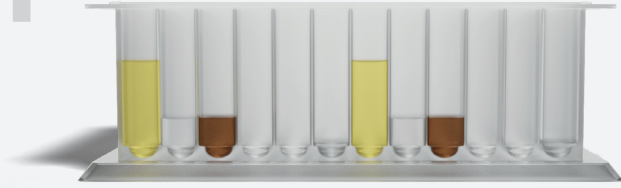


7.2 Eluates can be used directly in Kylt® Salmonella spp. kit

PROTOCOL AT A GLANCE

Kylt® Salmonella Purification HTP on Kylt® Purifier 48

1

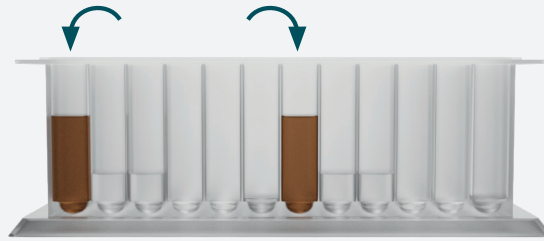


- Add 500 µl enriched samples to column 1 and 7.
- Add Wash Solution I to columns 2 and 8.
- Add Wash Solution II to columns 3 and 9.
- Add Elution Buffer to columns 6 and 12.

2



3.1
Mix Binding Reagent

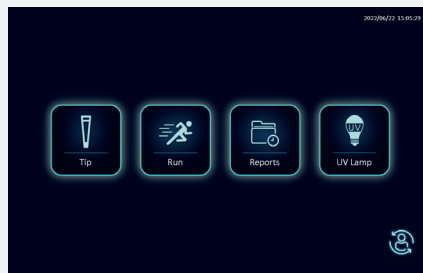


2.2
Add 750 µl Binding Reagent to each used well in columns 1 and 7.

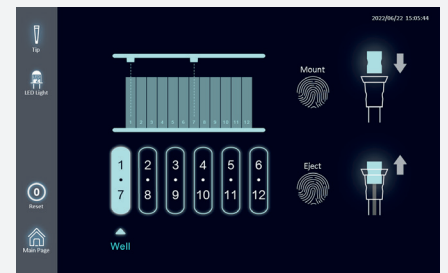
3



3.1
Turn on Kylt® Purifier 48 and log in.

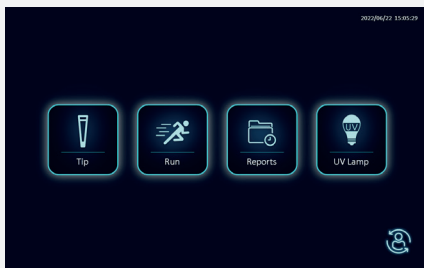


3.2
Choose Tip.

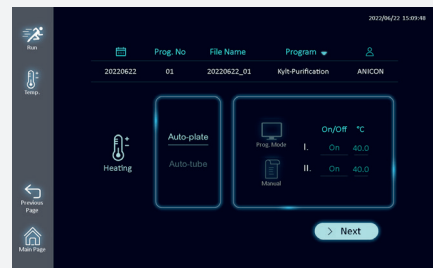


3.2
Load Tips as needed, remove Spin Tip Plate.

4



4.1
Click "Home" then choose "Run" and select the Kylt Salmonella program.

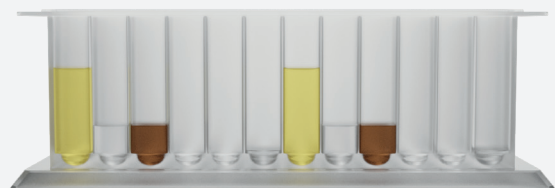


4.2
Load the prepared plate on the chosen position and follow the next steps.

5



5.1
Once protocol is finished, unload the Kylt® Purifier 48.

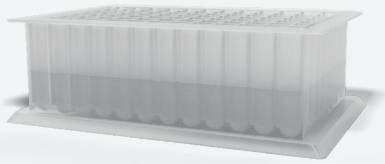


5.2
Eluates in columns 6 / 12 can be used directly in Kylt® Salmonella spp. kit

PROTOCOL AT A GLANCE

Kylt® Salmonella Purification Prefilled 96 on Kylt® Purifier

1

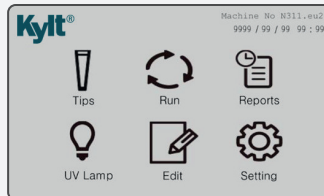


- Carefully remove the sealing foil from the Binding Plate.
- Add 500 µl enriched samples to each used well.
- Remove the sealing foils from the Wash Plates I and II, the Bead Plate and the Elution Plate.

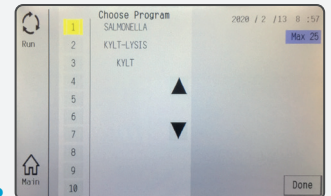
2



4.1
Turn on Kylt® Purifier

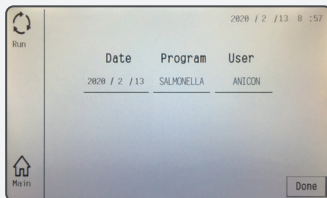


4.2
Click Run



4.3
Choose Salm Pref program

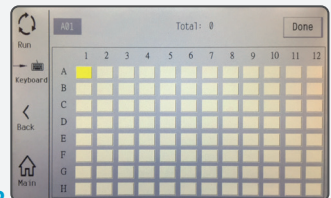
3



5.1
Confirm

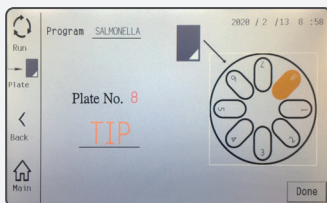


5.2
Enter Batch IDs if needed

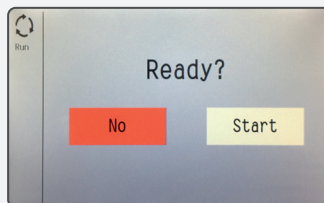


5.3
Enter sample data, if needed

4

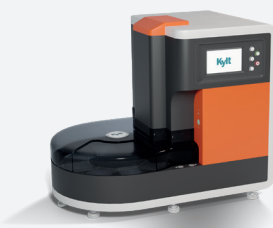


6.1
Load Kylt® Purifier as prompted



6.2
When all plates are properly loaded, start process

5



7.1
Once protocol is finished, unload Kylt® Purifier.

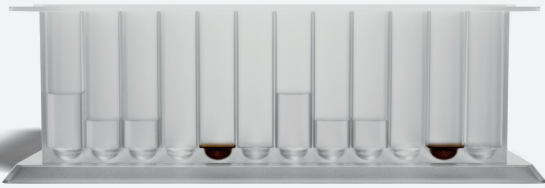


7.2
Eluates can be used directly in Kylt® Salmonella spp. kit

PROTOCOL AT A GLANCE

Kylt® Salmonella Purification Prefilled 48 on Kylt® Purifier 48

1



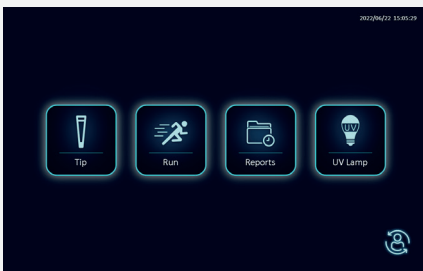
- Carefully remove the sealing foil of each used plate.
- Add 500 µl enriched samples to column 1 and 7.

2

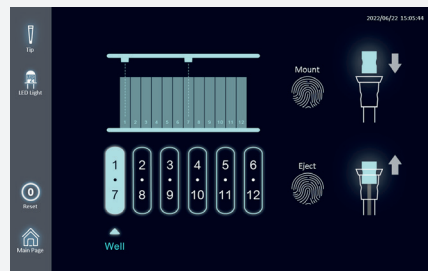


2.1
Turn on Kylt® Purifier 48 and log in.

3

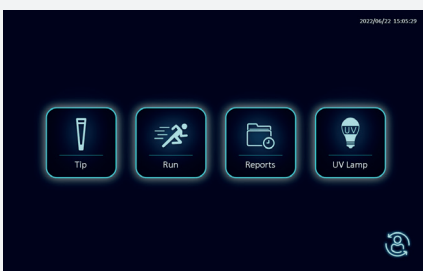


3.1
Choose Tip in the home screen

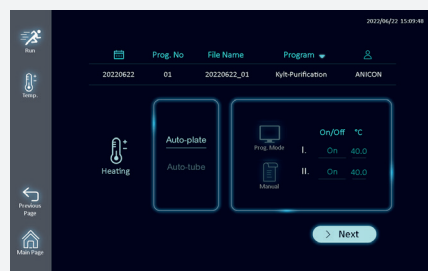


3.2
Load Tips for all positions of the used plate, remove Spin Tip Plate.

4



4.1
Choose "Run" and select the Salmonella Prefilled program.

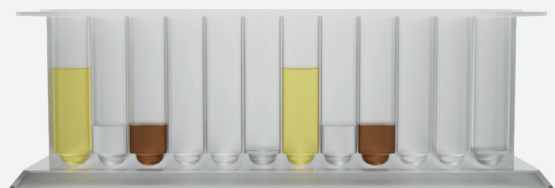


4.2
Load the prepared plate on the chosen position and follow the next steps.

5



5.1
Once protocol is finished, unload the Kylt® Purifier 48.



5.2
Eluates in columns 6 / 12 can be used directly in Kylt® Salmonella spp. kit