For veterinary diagnostics

Kylt®

Kylt® RNA / DNA Purification HTP

High Throughput Purification Kit for RNA and DNA from veterinary diagnostic samples

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Kylt®

Rev001, Feb 2022

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4x 96 purifications

Revision No.	Amendments
001	New Kit composition without ethanol.

A. Introduction

Kylt® RNA / DNA Purification HTP was developed to simplify the first crucial step in any molecular diagnostic application, which is the purification of target nucleic acids from common veterinary diagnostic samples.

Kylt® RNA / DNA Purification HTP is intended for use with automation equipment. It facilitates the purification of all relevant nucleic acids (viral RNA and DNA as well as procaryotic and eucaryotic RNA and DNA, e.g. from Bacteria and their host species) simultaneously from a broad range of sample matrices such as swabs, tissue, faeces, serum, plasma and other body fluids derived from different animal species and their environment. Samples from cultural processes e.g., bacterial pre-enrichments may also be used directly.

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.

B. Reagents and Materials

All kit components can be stored at room temperature and are stable for 15 months from date of production.

Reagent	Content 4 x 96 Purifications	Remark	GHS Classification
Lysis Solution	60 ml	Add appropriate amount of Internal Control RNA* (if applicable) and store appropriate aliquots at ≤ -18 °C.	
Proteinase K	5x 1600 µl	Store at +2 to +8 °C upon opening.	
Magnetic Beads	5x 1600 µl		
Binding Solution (concentrate)	1x 80 ml	Add 160 ml undenatured Ethanol ≥ 95 %.	
Wash Solution I (concentrate)	2x 156 ml	Add 94 ml undenatured Ethanol ≥ 95 % to each bottle.	
Wash Solution II (concentrate)	1x 48 ml	Add 192 ml undenatured Ethanol ≥ 95 %.	
Elution Buffer	60 ml		

*When using Kylt® Real-Time RT-PCR Detection Kits addition of 5 µl Kylt® IC-RNA per sample preparation is sufficient. Depending on the used samples the amount of Kylt® IC-RNA per sample can be adjusted based on an internal laboratory validation of the end user in line with the quality management system.

Apart from the disposables, the following devices and reagents are needed:

- Kylt® Purifier or other magnetic particle processor, liquid handling robot or magnetic separator.
- Disposables for the automation equipment used (e.g. plates, tip combs, pipette-tips).
- Heatable plate shaker.
- Vortex.
- Micropipettes covering volumes of 10 µl to 1000 µl. Filtered tips are obligatory.
- Homogenizer for tissue samples (e.g., bead tubes or mortar and pestle).
- Physiological sodium chloride solution (NaCl 0,9 %) or Kylt® Swab Wash Solution to process swabs or for tissue homogenisation.
- Undenatured Ethanol ≥ 95 % to prepare the Binding Solution and Wash Solution I and II.

C. General Recommendations

Great care should be taken to avoid degradation of purified RNA due to RNase contamination. RNases are very stable and active enzymes. Though they are inactivated by the components of the kit, any additional RNase-contamination should be avoided to ensure highest sensitivity in downstream assays.

In order to maintain an RNase-free environment the following precautions should be taken:

- Use of nuclease-free plastic consumables.
- Wearing gloves during the manual steps and while handling eluates.
- Keeping purified nucleic acids on ice or refrigerated at all times.

D. Sample preparation

- Pooling is possible, but needs to be verified with the subsequent detection system. A maximum of five individual samples or samples taken from five individuals, respectively, per purification is recommended.
- Swabs should be soaked in a sufficient volume of sterile sodium chloride solution (e.g., 0.9 %) for at least 5 minutes. Then, the sample is washed out thoroughly by pulse-vortexing and the supernatant is used.
- Alternatively, swab specimens may be washed in a mixture of water and Kylt® Lysis Solution (2:1.3 v/v). 330 µl of the supernatant (lysed swab sample) then only needs to be mixed with the Proteinase. This procedure inactivates viruses at an early stage in the protocol. The Kylt® Swab Wash Solution is available separately and consists of already diluted Kylt® Lysis Solution.
- Tissue samples should be homogenized thoroughly in sterile sodium chloride solution (e.g., 0.9 %). Centrifuge briefly to clear sample from large debris and use supernatant for the purification. Use up to 20 mg tissue per purification, e.g. homogenize 40 mg in 400 µl buffer, then use 200 µl supernatant.
- Material from cultural processes, such as cell culture supernatant, allantoic fluid or liquid samples with low host cell amount, such as bronchoalveolar lavage fluid, can be used directly.
- Fecal samples should be suspended in 10 times the volume of sterile sodium chloride solution (e.g., 0.9 %). Centrifuge briefly to clear from large debris. Use 200 µl supernatant.
- Snippets from sample submission cards (e.g. AniCard or FTA cards), loaded with the samples mentioned above, can be used per purification. Place them in a tube and add a sufficient amount of sterile sodium chloride solution (0.9 %). Proceed with the protocol through the lysis (add lysis solution and Proteinase K), and subsequently transfer 350 µl lysate (without the card punches) to the processing plate. Then add magnetic beads and binding solution. Depending on the size and number of the punches, adjust the volumes of sterile buffer, Lysis Solution, Proteinase K. Please be aware that increased wash-out volume dilutes the sample and may lead to diminished sensitivity.

E. Lysis Protocols

- Kylt® RNA / DNA Purification HTP is suitable for any diagnostic sample with the universal two-step protocol consisting of the lysis step and the subsequent purification step.
- It is possible to skip the lysis step and directly proceed to the binding step. This is only recommended for simple samples such as swab washouts or other samples with low content of PCR-inhibtiors and / or proteins, such as serum or plasma.

E. Application on the Kylt® Purifier

1. Lysis

Variant 1: Add 20 µl Proteinase K to each used well of the Kylt® Purifier Plate (mark Plate "Bind").

Variant 2: Add 20 µl Proteinase K and 20 µl Magnetic Beads to each used well of the Kylt® Purifier Plate (mark Plate "Bind").

- Add 200 µl sample and 130 µl Lysis Solution or add 330 µl lysed swab sample (in Kylt® Swab Wash Solution) to each used well of the Kylt® Purifier Plate.
- Start method "Kylt-Lysis" on the Kylt® Purifier.
- Load Kylt® Purifier Spin Tips on Position 8.
- Load Kylt® Purifier Plate containing the samples on position 1 and confirm the start of the lysis.

2. Preparing Wash Plates

- During lysis prefill four Kylt® Purifier Plates:
- Mark the first Kylt® Purifier Plate with "W-1" and fill each used well with 500 µl Wash Solution I.
- Mark the second Kylt® Purifier Plate with "W-2" and fill each used well with 500 µl Wash Solution I.
- Mark the third Kylt® Purifier Plate with "W-3" and fill each used well with 500 µl Wash Solution II.
- Mark the fourth Kylt® Purifier Plate with "ELUT" and fill each used well with 100 µl Elution Buffer.

3. Binding

- When the lysis is finished (about 15 min), start the protocol "Kylt-Purif" on the Kylt® Purifier.
- The Kylt® Spin Tips remain on Position 8. Confirm the placement.
- Remove the Kylt® Purifier Plate containing the samples.
- Add 20 µl Magnetic Beads to each used well of Kylt® Purifier Plate with the samples (if not already added before lysis (Variant 1)).
- Add 500 µl Binding Solution to each used well of Kylt® Purifier Plate with the samples.

4. Purification

- Replace the Kylt® Purifier Plate with the samples on position 1 and confirm the placement on the display.
- Load the Kylt® Purifier Plates W-1, W-2, W-3 and ELUT when prompted.
- Confirm the start of the purification method.

5. Completing the Purification

- When the protocol is complete after about 30 minutes, the plate ELUT is positioned in the front.
- Unload the plate and cover with adhesive foil and store refrigerated or use directly in a Real-Time PCR.
- Unload and discard the other plates and the Kylt® Purifier Spin Tips.

F. Manual Application

1. Lysis

Variant 1: Add 20 µl Proteinase K to one well of a 96 deep-well plate.

Variant 2: Add 20 µl Proteinase K and 20 µl Magnetic Beads to one well of a 96 deep-well plate.

- Add 200 µl sample and 130 µl Lysis Solution or add 330 µl swab washout (in Kylt® Swab Wash Solution) to the used well of the plate.
- Mix by pipetting up and down or shaking.
- Incubate for 10 minutes at +56 ± 2 °C.

2. Binding

- Resuspend Magnetic Beads.
- Add 20 µl Magnetic Beads to each used well. (not applicable for lysis according to variant 2, see point 2).
- Mix briefly by pipetting or shaking.
- Add 500 µl Binding Solution.
- Mix by pipetting, shaking or tip comb movement. Incubate 5 minutes.
- Separate Magnetic Beads (1 minute).
- Remove and discard supernatant.

3. Washing Step 1

- Add 500 µl Wash Solution I or transfer the Magnetic Beads to a new plate containing Wash Solution I.
- Mix briefly by pipetting or shaking.
- Incubate while keeping Magnetic Beads suspended (2 minutes).
- Separate Magnetic Beads (1 minute).
- Remove and discard supernatant.

4. Washing Step 2

- Repeat step 3.

5. Washing Step 3

- Add 500 µl 80% Ethanol or transfer the Magnetic Beads to a new plate containing Wash Solution II.
- Mix briefly by pipetting or shaking.
- Incubate while keeping Magnetic Beads suspended (1 minutes).
- Separate Magnetic Beads (1 minute).
- Remove and discard supernatant.

6. Drying

- Air dry magnetic beads (5 - 10 minutes, please verify).
- If a heatable shaker is used, heat plate to +56 ± 2 °C. Shaking further supports drying.
- Drying settings need to be adjusted to the instrument used.

6. Elution

- Add 50-200 µl Elution Buffer to the Magentic Beads pellet.
- Incubate 1 minute at +56 ± 2 °C with shaking.
- Separate Magnetic Beads (1 minute).
- Transfer eluate or remove Magnetic Beads.
- The eluate contains the purified RNA and DNA.
- The eluate can immediately be used in downstream applications such as Real-Time PCR.
- Long term storage of purified nucleic acids is recommended at ≤ -18 °C or at ≤ -70 °C.

G. Suggestions for Automation

1. Instruments

- This kit is ideally suited to be automated on the Kylt® Purifier and Kylt® Purifier 48.
- This kit can be automated on liquid handling robots or dedicated magnetic particle processors.

2. Protocols

- Protocols are available for the Kylt® Purifier, Kylt® Purifier 48, KingFisher® Instruments (BindIt 4.0) and Hamilton STAR Line (Venus 4), please inquire).

H. Troubleshooting

Observation	Possible Cause & Solution
Inhibition of PCR reaction	Ethanol carryover. Please check the bead pellet at the end of the drying step for residual ethanol. Prolong drying step in the protocol, if necessary. Sample was rich in humic acids or other inhibitors. Dilute eluate or repeat purification with less or diluted sample amount.

Product	Artikel No.	Content	Description
Kylt® Purifier	31436	1	Purification system for magnetic beads. Up to 96 samples can be processed in under 45 minutes.
Kylt® Purifier 48	31748	1	Purification system for magnetic Beads. Up to 48 samples can be processed in under 45 minutes.
Kylt® Purifier Spin Tips	31434	5	Plate with 96 separate spin tips, used by the Kylt® Purifer to mix the well contents by stirring. One set used per run.
Kylt® Purifier Plates	31435	20	Plates to be used for the several reactions and reagents in a nucleic acid purification kit. 4 - 5 plates used per run.
Kylt® Swab Wash Solution	31453	500 ml	Pre-diluted Kylt® Lysis Solution for washing out of dry swab samples.
Kylt® Salmonella Purification HTP	31433	4 x 96	Kit for the simple and automated purification of enriched samples for Salmonella detection.

Production:

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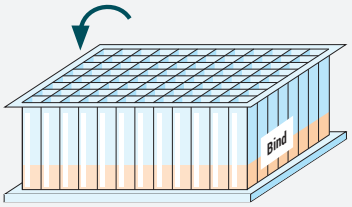
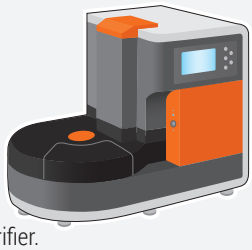
PROTOCOL AT A GLANCE

Kylt® RNA/DNA Purification HTP

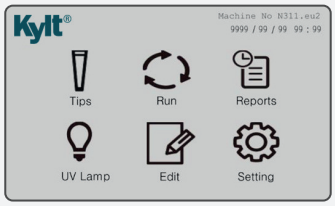
1



1.1
20 µl Proteinase and Magnetic 200 µl Sample, 130 µl Lysis Sol.

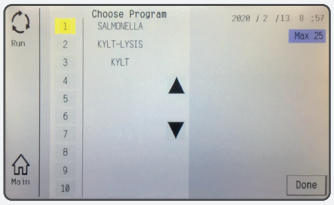


1.2
Turn on Kylt® Purifier.

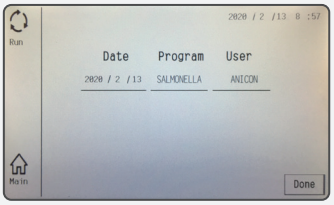


1.3
Choose Run.

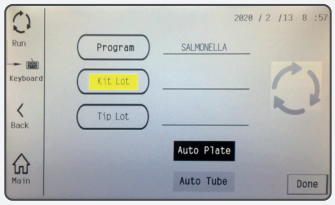
2



2.1
Choose Kylt-Lysis.

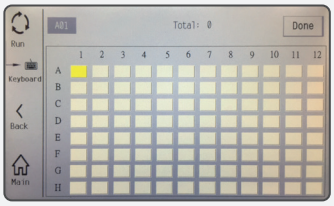


2.2
Confirm.

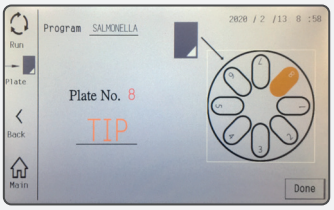


2.3
Enter batch-info, if needed.

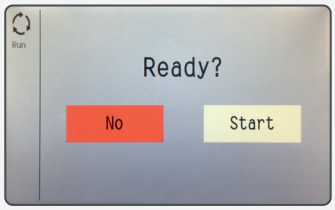
3



3.1
Enter sample-IDs, if needed.

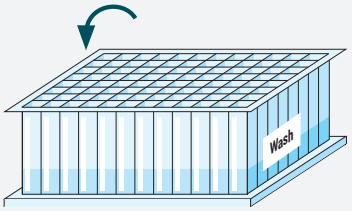


3.2
Load Kylt® Purifier as prompted.

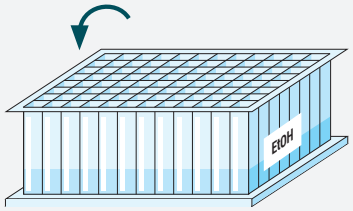


3.3
Confirm start.

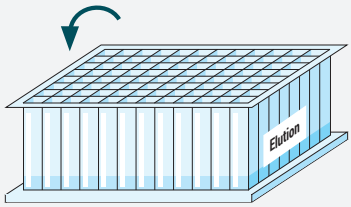
4



4.1
500 µl Wash Solution I into plates W-1 and W-2.

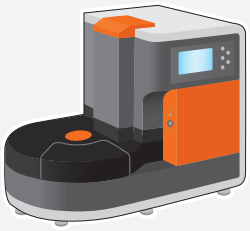


4.2
500 µl Wash Solution II into plate W-3.

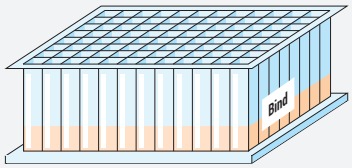


4.3
100 µl Elution Buffer into the Elution plate.

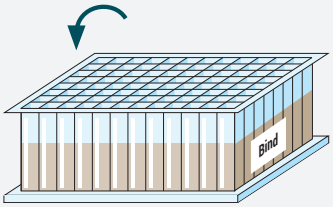
5



5.1
Wait for end of lysis.

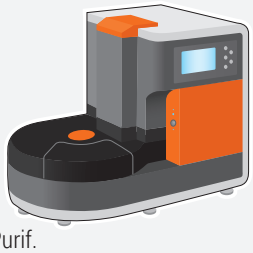


5.2
Remove plate "Bind".

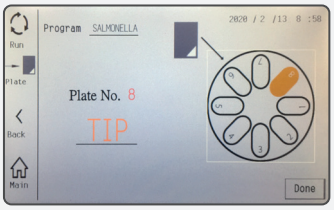


5.3
20 µl Magnetic Beads and 500 µl Binding Solution

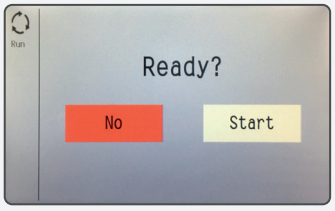
6



6.1
Start protocol Kylt-Purif.

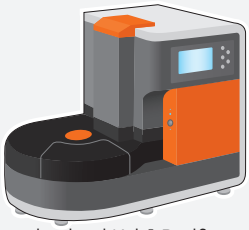


6.2
Load Kylt® Purifier as prompted.

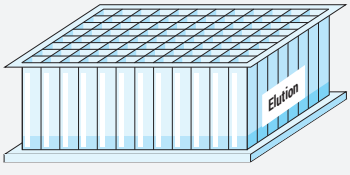


6.3
Confirm start.

7



7.1
Wait for end of the protocol and unload Kylt® Purifier.



7.2
Eluates are suitable for qPCR analysis.