For veterinary diagnostic use only.

Kylt®

Kylt® RNA / DNA Purification

Purification Kit for RNA and DNA from veterinary samples

www.kylt.eu

Rev006, Feb 2020

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5x 50 / 50 purifications

Revision No.	Amendments
006	Improvements to the graphics in the "Protocol at a Glance", Ordering Information added

A. Introduction

- Kylt® RNA / DNA Purification was developed to simplify the first crucial step in any molecular veterinary diagnostic application, which is the purification of target nucleic acids from common veterinary samples.
- Kylt® RNA / DNA Purification 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 animal species and their environment. Samples derived 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 18 months from date of production.

Reagent	Content 50 Purifications	Remark	GHS Classification
Lysis Solution	25 ml	Add appropriate amount of Internal Control RNA* (if applicable) and store appropriate aliquots at ≤ -18 °C.	
Liquid Proteinase K	600 µl	Store at +2 to +8°C upon opening.	
Wash Solution 1	30 ml	Add 9 ml Isopropanol before first use.	
Wash Solution 2	6 ml	Add 24 ml non-denatured Ethanol ≥96% before first use.	
Elution Buffer	30 ml		
Kylt® Binding Columns	50 pieces		
Collection Tubes	3 x 50 pieces		
Lysis & Elution Tubes	2 x 50 pieces		

The article No. 31314 (5x 50) consists of 5 boxes of 31315.

*When using Kylt® Real-Time RT-PCR Detection Kits or Reagents 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:
  - Table top micro-centrifuge (speed at least 13000 x g)
  - Vortex
  - Micropipettes covering volumes of 10 µl to 1000 µl. Filtered tips are recommended.
  - Heat Block set to +70 ± 2 °C
  - Homogenizer for tissue samples (e.g., bead tubes or mortar and pestle)
  - Physiological sodium chloride solution (NaCl 0,9 %) or 0,1x TE buffer to wash out swabs or homogenize other samples.
  - Isopropanol (molecular biology grade, to prepare Wash Solution 1)
  - 96-100% non-denatured Ethanol (to prepare binding and Wash Solution 2)

C. General Recommendations

- Do not interrupt the extraction and work quickly.
- Great care should be taken to avoid degradation of purified RNA due to RNase contamination. RNases are very stable and active enzymes, which are difficult to inactivate. In order to maintain an RNAse-free environment the following precautions should be taken:
  - Use of sterile nuclease-free plastic material including tubes and tips.
  - Wear protective gloves at all times and change frequently, especially upon contamination.
  - Keep tubes closed whenever possible.
  - While handling, it is recommended to keep purified nucleic acids on ice or refrigerated.
  - For long term storage, purified nucleic acids should be stored at ≤ -18 °C or at ≤ -70 °C.

D. Protocol *(> see also Protocol At A Glance at the end of this Direction For Use)*

1. Sample preparation

- Pooling of a maximum of five individual samples or samples taken from five individuals, respectively, per purification is recommended.
- Swabs should be pooled in a sufficient volume of sterile buffer (e.g., Normal Saline or 0.1 x TE) and soaked for an adequate period of time. Then, the sample is washed out thoroughly by pulse-vortexing and the supernatant is used. Alternativeyl swabs may directly be emerged in Lysis solution.
- Tissue samples should be homogenized thoroughly in sterile buffer (see above). Centrifuge briefly to clear sample from large debris and use supernatant for further analysis. Use up to 20 mg tissue per purification, e.g. homogenize 40 mg in 400 µl buffer, then use 200 µl supernatant. Loading of too much tissue may harm effectiveness of overall methodology by clogging of the membrane.
- Material from cultural processes, such as cell culture supernatant or allantoic fluid and 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 buffer. Centrifuge briefly to clear from large debris. Use 200 µl supernatant.
- Up to 5 punches from FTA cards, loaded with the samples mentioned above, can be used per purification. The diameter of each punch should not exceed 5 mm. Place them in a tube and add 200 µl sterile buffer (see above). Proceed with the protocol through the lysis, and transfer the lysate (without the FTA card punches) to a new tube, prior to adding Ethanol. (If several punches are used, adjust the volumes of sterile buffer, Lysis solution, Proteinase K and Ethanol accordingly; use multiple loading steps, i.e. 600 µl per each step.)

2. Lysis

- Add 200 µl Lysis Solution to a Kylt® Lysis Tube. If Kylt® Internal Control RNA (IC-RNA) is to be used please refer to chapter B. and the direction for use of the respective Kylt® Real-Time RT-PCR Detection kit.
- Add 200 µl sample. Avoid transferring sample material that could subsequently lead to clogging of the Kylt® Binding Column, e.g. tissue or swab particles.
- Add 10 µl Liquid Proteinase K to a lysis tube (provided).
- Pulse-Vortex and spin down briefly.
- Incubate 5 minutes at room temperature, then 5 minutes at +70 ± 2 °C.
- Let sample cool down for 2 minutes.

3. Binding

- Add 200 µl Ethanol 96%.
- Pulse-Vortex to mix thoroughly, spin down briefly.
- Transfer mixture to Kylt® Binding Column.
- Centrifuge 1 minute at 10000 g.
- Discard Collection Tube with flow-through. Attach new Collection Tube (provided).

4. Washing

- Add 500 µl Wash Solution 1 to the Kylt® Binding Column.
- Centrifuge 1 minute at 10000 g.
- Discard Collection Tube with flow-through. Attach new Collection Tube (provided).
- Add 500 µl Wash Solution 2 to the Kylt® Binding Column.
- Centrifuge 2 minutes at maximum speed.
- Discard Collection Tube with flow-through. Avoid carry-over of residual wash buffer. Attach Elution Tube (provided).

5. Elution

- Add 100 µl Elution Buffer directly to the membrane without touching it with the pipet tip.
- Incubate 1 minute at +70 ± 2 °C.
- Centrifuge 1 minute at maximum speed.
- Discard the Kylt® Bindung Column. The eluate contains the purified RNA and DNA and can immediately be used for further downstream applications such as Real-Time PCR.

E. Troubleshooting

Observation	Possible Cause & Solution
Lysate does not pass membrane	Repeat centrifugation at full speed for 2 minutes. If membrane is still clogged, repeat purification with new sample.
Inhibition of PCR reaction	Ethanol carryover. Prolong centrifugation step after Wash Solution 2. When removing collection tube, take extra care, not to transfer flow-through to binding column. Sample was rich in humic acids or other inhibitors. Dilute eluate.

F. Ordering information

For a fast and efficient service please send your order to orders@kylt.eu and please provide the following information:

- Delivery address
- Invoice address
- Purchaser contact telephone number
- End user name and telephone number (if different)
- Purchase order number
- Product name and cataloge number
- Quantity and size of products
- Indicate if your account is VAT exempt

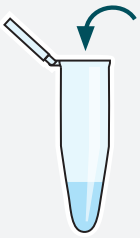
Production:

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

PROTOCOL AT A GLANCE

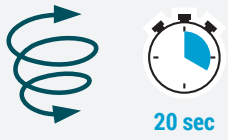
Kylt® RNA / DNA Purification

1



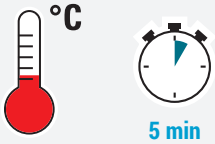
200 µl Lysis Solution
+ 200 µl sample
+ 10 µl Proteinase K

2

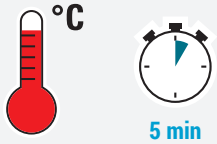


2.1
Vortex 20 s

2.2
Spin down 5 s



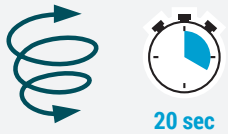
2.3
Incubate 5 min
room temperature



2.4
Incubate 5 min **70±2°C**

3

3.1
Add 200 µl Ethanol



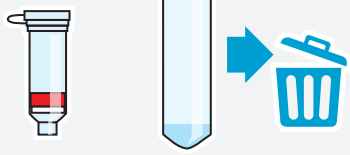
3.2
Vortex 20 s

3.3
Spin down 5 s

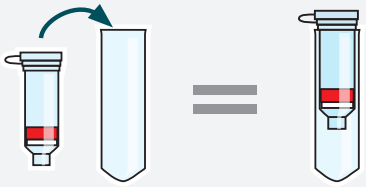
3.4
Transfer to
Kylt® Binding Column

3.5
Spin 10000 g, 60 s

4



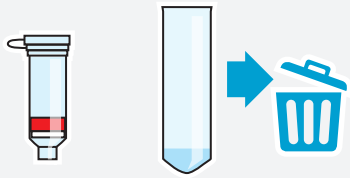
4.1
Discard Collection Tube and flow-through



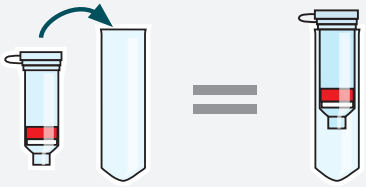
4.2
Place Kylt® Binding Column on new Collection Tube
Add 500 µl **Wash Solution 1**

4.3
Spin 10000 g, 60 s

5



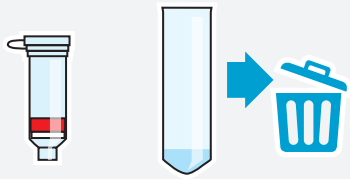
5.1
Discard Collection Tube and flow-through



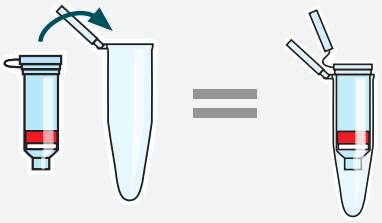
5.2
Place Kylt® Binding Column on new Collection Tube
Add 500 µl **Wash Solution 2**

5.3
Spin at **max. speed**, 2 min

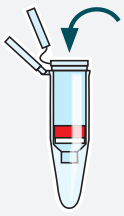
6



6.1
Discard Collection Tube and flow-through

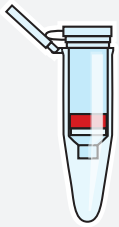


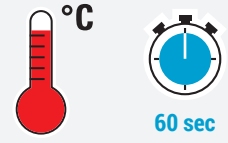
6.2
Place Kylt® Binding Column on Elution Tube



6.3
Add 100 µl Elution Buffer

7





7.1
Incubate 60 s **70±2°C**

7.2
Spin at **max. speed**, 60 s

7.3
Discard Kylt® Binding Column and close elution tube

AC.R/DNA.02, Rev006, February 2020