Kylt® DNA Extraction-Mix II

DNA Preparation Reagent

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DNA Preparation Reagent

A. General

- Kylt® DNA Extraction-Mix II is intended for simplified and economic DNA extraction of bacterial DNA from swabs, pure bacterial isolates and bacterial enrichments.

B. Reagents and Materials

- Kylt® DNA Extraction-Mix II is available as follows:

Reagent	Colour code of lid	Article No 31398	Storage conditions
Kylt® DNA Extraction-Mix II	◯ white	1x 20 ml	+2 °C to +8 °C

- After receipt, the Kylt® DNA Extraction-Mix II is immediately stored at +2 °C to +8°C.
- The Kylt® DNA Extraction-Mix II is to be used within the indicated shelf life (see label on the outer packaging).

C. Equipment and Reagents not included

- Apart from the disposables, the following further devices are needed and are not included in the product:
  - Vortex
  - Micropipettes covering volumes of 1 µl to 1000 µl
  - Centrifuge
  - Magnetic stirrer
  - Dry heating block
- We recommend the exclusive use of certified Nuclease-free disposables as well as powder-free protective glove. Please wear gloves during the entire experimental procedure. Gloves need to be changed frequently, especially after spillage or suspected contaminations.

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

- The overall protocol of the analysis consists of the following main workflow:

  1. Sample Preparation
  2. Kylt® DNA Extraction
  3. if applicable Real-Time or conventional PCR data analysis

- We recommend proceeding through the protocol without interruption to avoid potential degradation of the processed samples and reagents.

1. Sample Preparation

- We recommend pooling of at most five samples or samples from five individuals, respectively, per DNA preparation.
- Pool swabs in a sufficient volume of sterile buffer (e.g. 1 ml of Normal Saline or 0.1 x TE), let the swabs soak for an adequate period of time and finally wash out the swabs by thorough pulse-vortexing. The washed out supernatant is used for DNA preparation. For Kylt® DNA Extraction the supernatant is (fully) transferred to a conical screw cap tube. Small swabs may directly be immersed in Kylt® DNA Extraction-Mix II.
- Material derived from cultural processes, i.e. pure or mixed colony material or (pre-)enrichments, is directly transferred into a tube for Kylt® DNA Extraction, such as conical screw cap tube. For this, a little amount of a single colony is picked with a sterile loop wire or sterile pipette tip and transferred to the tube.

2. Kylt® DNA Extraction

- Pre-heat heating block to set temperature of +100 °C, the block must have an actual temperature of +100 °C ± 3 °C at first use.
- The swab washout or cultural material in the conical screw cap tube is pelleted by centrifugation at 10.000 g to 12.000 g for 10 min. For pure colony material this and the next step does not apply.
- Remove the supernatant (if applicable) using a 1000 µl pipette tip (not by decantation) and discard it. Take care not to remove pellets that may at times be hardly visible or invisible at all. In these cases, preferably do not aspirate the supernantant completely.
- The Kylt® DNA Extraction-Mix II needs to be stirred on a magnetic stirrer, to be used as a homogenous suspension. The pellet derived from swab washout is resuspended by repeated up-and-down pipetting with 20 µl to 200 µl of Kylt® DNA Extraction-Mix II. The volume of Kylt® DNA Extraction-Mix II for resuspension of the pellet should be chosen as small as possible, depending on the size of the pellet (e.g. for hardly visible pellets of tracheal swabs use 50 µl), to minimize dilution effects. In case of pure or mixed colony material a large amount of target bacterial DNA is expected, thus use 500 µl of Kylt® DNA Extraction-Mix II and thoroughly resuspend the transferred material. Avoid formation of bubbles and aerosols.
- Screw cap tight, vortex thoroughly and incubate for 10 min to 15 min at +100 °C ± 3 °C.

- Vortex sample thoroughly and centrifuge at 10.000 g to 12.000 g for 5 min; the supernatant is the final DNA Extract and can be used for Real-Time or conventional PCR immediately. Short-term storage (few hours) of the DNA extract at +2 °C to + 8 °C is possible. For long-term storage of the DNA extract at ≤ -18 °C, take supernatant and transfer to new (screw cap) tube. Before next use in Real-Time or conventional PCR incubate DNA extracts that were stored at ≤ -18 °C for few minutes at +100 °C ± 3 °C, vortex and spin down.

3. Real-Time or conventional PCR and data analysis

- For detailed information about the Reaction Setup and data analysis of Real-Time or conventional PCR please see Direction For Use of the respective Kylt® product.

E. Related and Accessory Products

Product	Article No	Reactions	Description
Kylt® RNA/DNA Purification	31314 / 31315	250 / 50	Combined RNA and DNA purification from veterinary samples

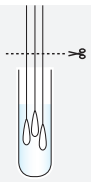
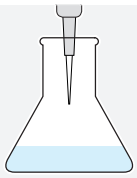
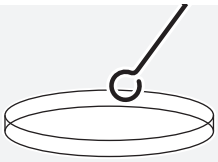
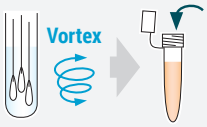
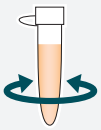
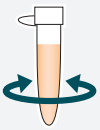
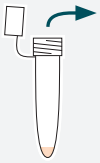
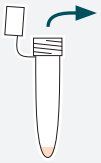
Production:

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

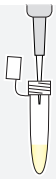
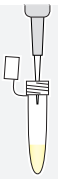
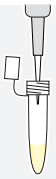
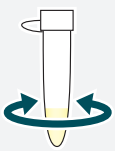
PROTOCOL AT A GLANCE

Kylt® DNA Extraction-Mix II

SAMPLE HANDLING

	swab samples	cultural material	pure or mixed cultural material
1	 pool max. 5 samples in tube with saline (0.9%) or 0.1 x TE	 transfer 1 ml of supernatant with a pipette	 pick a little amount of a single colony with a sterile loop wire
2	 2.1 wash by vortexing 2.2 transfer total supernatant	 transfer into screw cap tube	 transfer into screw cap tube
3	 **10.000-12.000 g 10 min**	 **10.000-12.000 g 10 min**	
4	 Discard supernatant	 Discard supernatant	

DNA EXTRACTION

5	 Add **20–200 µl** DNA Extraction-Mix II + **20–200 µl**	 Add **200 µl** DNA Extraction-Mix II + **200 µl**	 Add **500 µl** DNA Extraction-Mix II + **500 µl**
6	 **Vortex**	Mix by vortexing	
7	 **10-15 min 100°C**	Incubation 10-15 min 100°C	
8	 **Vortex**	Mix by vortexing	
9	 **10.000-12.000 g 5 min**	10.000–12.000 g 5 min	

AC.DNA-ExM-II.02, Rev001, December 2017