



V For *in vitro* use only.

Kylt[®]

Kylt[®] DNA Extraction-Mix III

DNA Preparation Reagent

www.kylt.eu

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DNA Preparation Reagent

A. General

- Kylt® DNA Extraction-Mix III is intended for simplified and economic DNA extraction from hard to lyse pure bacterial isolates or bacterial enrichments (e.g. *Listeria monocytogenes*).

B. Reagents and Materials

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

| Reagent | Colour code of lid | 100 Reactions Article No 31404 | Storage conditions |
|-----------------------------|--------------------|-----------------------------------|--------------------|
| Kylt® DNA Extraction-Mix II | ○ white | 1x 18 ml | +2 °C to +8 °C |
| Lysis Beads | ○ transparent | 1x 8 g | +2 °C to +8 °C |

- After receipt, the components are immediately stored at +2 °C to +8°C.
- The components are to be used within the indicated shelf life (see box label).

C. Equipment and Reagents not included

- Apart from the disposables, the following further devices are needed and are not included in the product:
 - Stomacher®, masticator or equivalent for homogenizing test samples
 - Incubator, capable of maintaining +30 °C ± 1 °C
 - Micropipettes covering volumes of 1 µl to 1000 µl
 - Centrifuge
 - Table top microcentrifuge, Vortex and Magnetic stirrer
 - Bench top homogenisator for cell disruption
 - 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

- The overall protocol of the analysis consists of the following main workflow:
 1. Cultural Pre-Enrichment
 2. 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. Cultural Pre-Enrichment

- Please refer to the according regulations or the specific in-house method.
- When using Kylt® DNA Extraction-Mix III in combination with Kylt® *Listeria monocytogenes*, please refer to its Direction For Use for detailed information about the cultural pre-enrichment.

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 final Lysis Buffer (DNA Extraction-Mix II and Lysis Beads) is stirred on a magnetic stirrer, it must be used as a homogenous suspension.

Note: Gently shake the final Lysis Buffer by hand first to resuspend the beads. Stir the final Lysis Buffer on a medium moving magnetic stirrer and pipette the reagent during stirring. Use pipettes with a wide tip to allow pipetting of the particles in the homogenized lysis buffer.

- The aliquoted pre-enrichment in the conical screw cap tube is pelleted via centrifugation at 10.000 g to 12.000 g for 5 min.
- Remove the supernatant using a 1000 µl pipette tip (not by decantation) and discard it.
- The pellet is resuspended by repeated up-and-down pipetting with 250 µl of the final Lysis Buffer. Use a 1000 µl pipette with filtered tips. Avoid formation of bubbles and aerosols.
- Place the screw cap tube in a cell disruptor for 3 min ± 1 min. In case of using instruments with higher efficiency reduce the time according to the manufacturer's instructions.
- Incubate the sample 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 DNA extract and can be used for Real-Time 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 to -20 °C, take supernatant and transfer to new (screw cap) tube. Before next use in Real-Time PCR incubate DNA extracts that were stored at -18 °C to -20 °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 please see Direction For Use of the respective Kylt® product.

E. Related and Accessory Products

| Product | Article No | Reactions | Description |
|------------------------------|---------------|-----------|--|
| Kylt® List mono | 31013 / 31014 | 100 / 25 | Detection of <i>Listeria monocytogenes</i> in food and feed samples. |
| Kylt® Listeria monocytogenes | 31651 / 31652 | 100 / 25 | Detection of <i>Listeria monocytogenes</i> in veterinary samples. |
| Kylt® RNA/DNA Purification | 31314 / 31315 | 250 / 50 | Combined RNA and DNA purification from veterinary samples |

F. Ordering information

For a fast and efficient service please send your order to orders.kylt-de@san-group.com 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 catalogue number
- Quantity and size of products
- Indicate if your account is VAT exempt

Production:

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Development, manufacturing and distribution of Kylt® *In-Vitro* Diagnostica is certified according to ISO 9001:2015.

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