For *in vitro* Diagnostics only.

Kylt®

Kylt® SARS-CoV-2 Complete RTU

Real-Time RT-PCR Detection

www.kylt.eu

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A. General

- Kylt® SARS-CoV-2 Complete RTU kits are intended for the combined detection of SARS-CoV-2 in two independent specific target genes in one reaction. The kits are for in vitro diagnostic purposes and suitable for the analysis of samples from humans, such as naso-/oro-pharyngeal or rectal swabs, bronchoalveolar lavage fluid, sputum as well as environmental samples.
- The qualitative testing with Kylt® SARS-CoV-2 Complete RTU kits is based on a triplex Real-Time RT-PCR: In one reaction setting, the RNA target sequences for SARS-CoV-2 (IP4- and S-gene) as well as for the endogenous control (beta-Actin) are reverse transcribed (Reverse Transcription (RT)) and amplified in parallel with respective primer pairs in the Polymerase Chain Reaction (PCR). Amplified target gene fragments are detected via fluorescently labeled probes during the PCR reaction in real-time (Real-Time PCR). The probes specific for detection of amplified SARS-CoV-2 and the endogenous control target genes are labeled with fluorescent dyes FAM, Cy5 and HEX, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of all three individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run, the SARS-CoV-2-specific status of a sample can be evaluated in the end. This way, results can be achieved within a few hours after sample receipt.
- These kits were developed for use by trained laboratory personnel following standardized procedures. This Direction For Use must be followed strictly. For in vitro diagnostic use only.
- The setup used for the specific detection in this kit includes a published setup listed by the World Health Organization (WHO).

IVD

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49685 Höltinghausen
Germany

REF
- 31469 for 100 reactions
- 31470 for 25 reactions

B. Reagents and Materials

- The following Kylt® SARS-CoV-2 Complete RTU kits are available and comprise the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31467 REF	25 Reactions Article No 31468 REF	Store at
Reaction-Mix	orange	4 x 450 µl	1 x 450 µl	≤ -18 °C
Positive Control	red	4 x 50 µl	2 x 50 µl	≤ -18 °C
Negative Control	blue	1 x 1 ml	1 x 1 ml	≤ -18 °C

- After receipt, the components are immediately stored at ≤ -18 °C. Avoid repeated freezing and thawing of all the reagents and keep them thawed as shortly as possible. If occasional processing of only a few samples is expected you may prepare appropriate aliquots of reagents before storage at ≤ -18 °C. Prepare aliquots in such a way that freeze-thaw-cycles are reduced to a maximum of three. The Negative Control can alternatively be stored at +2°C to +8°C.
- The components are to be used within the indicated shelf life (see box label). The components of different batches may not be mixed.
- The Reaction-Mix needs to be stored protected from abundant light. Do not expose to direct (sun)light.

C. Equipment and Reagents not included

- This detection method can be used on all commercially available Real-Time PCR thermal cyclers that detect the emitted fluorescence of the fluorescent dyes FAM, Cy5 and HEX (emission 520, 670 and 550 nm, respectively). Note that default normalization option against ROX (e.g. using ABI cyclers) must be deactivated.
- Apart from the disposables, the following further devices are needed but are not included in the Kylt® SARS-CoV-2 Complete RTU kits:
  - RNA preparation kit / protocol (e.g. Kylt® RNA / DNA Purification products)
  - Table top microcentrifuge
  - Vortex
  - Micropipettes covering volumes of 1 µl to 1000 µl
  - Centrifuge for PCR tubes or plates
- Accessory Kylt® products: see chapter H “Related and Accessory Products”.
- We recommend the exclusive use of certified Nuclease-free disposables as well as powder-free protective gloves. Please wear gloves during the entire experimental procedure. Gloves need to be changed frequently, especially after spillage or suspected contaminations.

D. Control Reactions

- The Positive Control allows for control of the specificity and efficiency of the reagents and the reaction itself, including the performance of the Real-Time RT-PCR and of the Real-Time PCR thermal cycler.
- The Negative Control allows for exclusion of contaminations. The sample testing is only valid if both, Positive and Negative Controls, are used and valid in every Real-Time RT-PCR run.
- The Internal Control is based on detection of beta-Actin RNA, which is ubiquitous in the cells of the host that the sample is derived from. The beta-Actin RNA target gene is co-amplified (channel HEX) with every single reaction and allows for evaluation of sufficient sampling, sample storage and shipment, sample preparation and the Real-Time RT-PCR run itself.
- It is recommended to run one or more RNA Isolation Control(s) (RIC) per set of RNA preparation, depending on the total number of samples processed at once. The RIC is a "mock sample" composed of the plain sterile buffer used for raw sample processing. It is randomly placed between the samples, processed like a normal sample and allows to detect potential contaminations of the reagents used (additionally to the Negative Control reaction) as well as for the detection of potential carryover contaminations between individual samples, e.g. during the RNA preparation process.

E. 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. RNA Preparation
3. Reaction Setup, Reverse Transcription and Amplification (Real-Time RT-PCR)
4. Data Analysis – Validity and Qualitative Result

- We recommend proceeding through the protocol without interruption to avoid potential degradation of the processed samples and reagents. If necessary, you may store the final RNA preparation at ≤ -18 °C until further processing. Avoid repeated freezing and thawing of the RNA preparations.

1. Sample Preparation

- Let swabs soak in a sufficient volume of sterile buffer (e.g. 1 ml of Normal Saline or 0.1 x TE) for an adequate period of time and finally wash out the swabs by thorough pulse-vortexing.
- The supernatant is used for RNA preparation.
- Small swabs may directly be immersed in lysis buffer, if applicable.

2. RNA Preparation

a) Kylt® RNA/DNA Purification products

- All kinds of sample matrices including swabs may be processed with Kylt® RNA/DNA Purification products (please refer to chapter H "Related Products").
- For detailed information on the RNA preparation process, please refer to the respective Direction For Use.

b) Alternative methods

- All kinds of sample matrices including swabs may be processed with appropriate RNA preparation kits or appropriate in-house methods.
- For detailed information on the RNA preparation process, please refer to the Direction For Use or Standard Operating Procedure of the specific kit or in-house method, respectively.

3. Reaction Setup and Amplification (Real-Time RT-PCR)

- Before each use, briefly vortex and spin down the Reaction-Mix and Negative Control.
- To determine the total number of reactions needed, count the number of samples and add two more for the Negative Control and the Positive Control.
- The Reaction-Mix is ready-to-use, add 16 µl to each of the PCR tubes or plate wells ("cavities").
- Keep exposure of the Reaction-Mix to (sun)light as short as possible and return it back to appropriate storage temperature right after application. Avoid the formation of bubbles when pipetting samples and controls.
- Add 4 µl of the Negative Control to the corresponding cavity and seal it individually, if possible.
- Add 4 µl of each RNA preparation to the corresponding cavities and seal them individually, if possible.
- To minimize risk of potential cross-contaminations, 4 µl of the Positive Control are added to the corresponding cavity after all previous samples and control reactions are set up. Before each use, briefly vortex and spin down the rehydrated Positive Control (see also chapter B "Reagents and Materials").
- If not already done, finally seal the cavities. It is recommended to briefly spin them down before the start of the Real-Time RT-PCR run.
- Place the cavities in the Real-Time PCR thermal cycler and run the test with Kylt® Profile I as given below.

Kylt® Profile I				
Step No	**Description**	**Temperature**	**Duration**	
1	Reverse Transcription	50 °C	10 min	
2	Activation of Polymerase	95 °C	1 min	
3	Denaturation	95 °C	10 sec	42 cycles
4	Annealing & Extension	60 °C	1 min	42 cycles
5	Fluorescence Detection	channels FAM, Cy5 and HEX		42 cycles

- Kylt® Profile I allows for combined run of this and most other Kylt® RT-qPCR detection methods as well as Kylt® PCR detection products.
- In the event of a combined Real-Time (RT-)PCR run, make sure all necessary channels are detected.
- Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.

4. Data Analysis – Validity and Qualitative Result

General

- The amplification data can be processed automatically using the specific software tool of your Real-Time PCR thermal cycler. Alternatively, the threshold can be set manually considering the following directions: The threshold should cross the FAM-, Cy5- and HEX-curves in the exponential phase of the PCR reaction (best visible as a linear slope when the Y-scaling is set to logarithmic). By setting the threshold, the crossing points with the HEX-, Cy5- and FAM-curves determine the respective cycle threshold (Ct), which is negatively correlated with the initial concentration of copies of the target genes in the Real-Time RT-PCR reaction.
- Only curves with the typical exponential amplification, meaning the curve of the raw data shows a flat baseline at the beginning, followed by a clear exponential phase and optionally reaching a plateau phase should be regarded positive.
- The actual test analysis starts with the validity check of the entire Real-Time RT-PCR run. Afterwards, by means of the Internal Control the validity of each sample reaction and its true test result can be verified according to the Ct-value of the Internal Control channel (HEX). Finally, the SARS-CoV-2-specific status of each sample is analyzed (FAM & Cy5).

Test Evaluation

- The **Real-Time RT-PCR test run** is only **valid,** if the FAM-, Cy5- and HEX-curves of the Negative Control are negative (Ct > 35 for HEX-channel) and the FAM-, Cy5- and HEX-curves of the Positive Control are positive. For a valid test the FAM-, Cy5- and HEX-Ct-values of the Positive Control have to be > 15 and ≤ 35.
- If one or more of a RNA Isolation Control (RIC(s)) is processed, its FAM-, Cy5- and HEX-curves must be negative (Ct > 35 for HEX-channel).

Target	Channel	Signal				
Internal Control	HEX	positive	positive / negative	positive / negative	positive / negative	negative
SARS-CoV-2 (IP4-gene)	FAM	negative	positive	negative	positive	negative
SARS-CoV-2 (S-gene)	Cy5	negative	positive	positive	negative	negative
The sample is SARS-CoV-2		**negative**	**positive**	**positive**	**positive**	**inhibited**

- A **sample is negative for SARS-CoV-2,** if its HEX-curve is positive (Ct ≤ 35), but its FAM- and Cy5-curves are negative.
- A **sample is positive for SARS-CoV-2,** if its FAM- or Cy5-curves are positive or if both curves (FAM and Cy5) are positive (Ct ≤ 42), independent of the HEX-curve.
- A **sample is inhibited,** if neither the FAM- and Cy5-curves nor the HEX-curve are positive.
- **Recommendation:** In the case of an inhibited sample the test may be repeated with a dilution of the RNA preparation at e.g. 1:4. The Negative Control is used as the diluting agent. Preferably, the entire RNA preparation process is repeated with a new sample using Kylt® RNA/DNA purification products or appropriate alternative.
- Convenient and reliable sample data entry, Real-Time PCR start, final qualitative analysis and documentation can be conducted with the Kylt® Software, please inquire.

5. Product Limitations

- Users must be trained and familiar with this product and procedures prior to application.
- Results generated by this product must be interpreted in context to clinical or further laboratory findings. It is the user's responsibility to verify performance for any procedures applied in their laboratory that are not covered by the performance data shown below.
- A negative result does not confirm status of non-infection, as results depend on appropriate specimen collection, viral load in specimen above Limit of Detection and absence of inhibitors, which would lead to invalid results. By use of the Internal Control, this product allows testing for the presence of PCR inhibitors and consequently minimizes the risk of false negative results.

F. Performance Characteristics

1. Analytical Sensitivity

- The Limit of Detection is < 3 copy per µl of RNA eluate using a quantified field sample eluate (oropharyngeal swab of infected person) for both channels.

2. Analytical Specificity

2.1 Inclusivity

2.1.1 In-silico Testing

- Determination of the epidemiological sensitivity is based on in silico testing using the nucleotide sequence database NCBI (National Center for Biotechnology Information). Oligonucleotide sequences (primers and probe) of the method were tested with regard to a cross-reaction or unspecific hybridization. To this end, the oligonucleotide sequences were aligned with the non-redundant data bank for DNA and RNA ("GenBank", NCBI) using Primer-BLAST and NBLAST.
- The assay detected all 3703 available full genome sequences of SARS-CoV-2 in-silico and several additional entries with partial sequences.

2.1.2 In-vitro Testing

- To further access the inclusivity of the methods, 52 field samples previously tested positive in a published reference method were tested with Kylt® SARS-CoV-2 Complete RTU, showing a perfect agreement. Also, reference strain 2019-nCoV/Italy-INMI1 was tested positive.

2.2 Exclusivity

- To address the exclusivity of the methods included in the kits, reference material of closely related Coronaviruses or pathogens commonly found in humans were tested with the method.

Strain	Result
Severe Acute Respiratory Syndrome Virus, isolate bat-SL-CoVZC45*	Not detectable
Middle East Respiratory Syndrome Virus, isolate KNIH/002_05_2015*	Not detectable
Bordetella parapeRTUssis**	Not detectable
Bordetella peRTUssis**	Not detectable
Chlamydia pneumoniae**	Not detectable
Coronavirus 229E**	Not detectable
Recombinant Coronavirus HKU1**	Not detectable
Recombinant Coronavirus NL63**	Not detectable
Recombinant Coronavirus OC43 strains 1 and 2**	Not detectable
Recombinant Human Metapneumovirus**	Not detectable
Human Rhinavirus**	Not detectable
Influenza A spp.**	Not detectable
Influenza A subtype H1**	Not detectable

Strain	Result
Influenza A subtype H1-2009**	Not detectable
Influenza A subtype H3**	Not detectable
Influenza B**	Not detectable
Mycoplasma pneumoniae**	Not detectable
Parainfluenza Virus 1**	Not detectable
Parainfluenza Virus 2**	Not detectable
Parainfluenza Virus 3**	Not detectable
Recombinant Parainfluenza Virus 4a**	Not detectable
Respiratory Synctical Virus**	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant 793b)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant QX)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant Q1)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant Var2)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant Israel 02)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant Massachusetts)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant Arkansas)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant D181)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant Italy02)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant D1466)***	Not detectable
Avian Coronavirus (Infectious Bronchitis Virus Variant D274)***	Not detectable
Bovine Coronavirus***	Not detectable

*Obtained from Integrated DNA technologies, Inc.

**Obtained as reference material from Microbiologics Ltd, St. Cloud, Minnesota USA 56303.

***Obtained from AniCon's internal reference strain collection.

- Furthermore, assays for both target genes combined in the Kylt® SARS-CoV-2 Complete RTU kit were analyzed in-silico for cross-reaction with other viruses using the nucleotide sequence database of the NCBI (National Center for Biotechnology). Therefore, the oligonucleotide sequences were tested in-silico with regard to cross-reaction and unspecific hybridization.
- The method does not cross react with other viruses especially not with other Sarbecoviruses such as SARS-CoV or further Coronaviridae and species of orders Alpha-, Beta-, Delta- or Gammacoronavirus, respectively.

3. Reproducibility

- To test the Intraassay Variance samples with defined CT-values were analysed in replicates in the same PCR run. The standard deviation of the replicates is < 0.5 CT values, the correlation coefficient is < 5 %.

4. Robustness

- To test the Interassay Variance samples with defined CT-values were analysed on different machines using different operators. The standard deviation of the samples is < 0.5 CT values, the correlation coefficient is < 5 %.

G. Symbols used on labels

IVD ▪ In Vitro Diagnostic Medical Device

REF ▪ Catalogue Number

▪ Manufacturer

H. Related and Accessory Products

Product	Article No	Reactions	Description
Kylt® SARS-CoV-2 Screening	31463 / 31464	100 / 25	Screening of samples for presence of Sarbecoviruses incl. the novel SARS-CoV-2
Kylt® SARS-CoV-2 Confirmation	31465 / 31466	100 / 25	Confirmation of SARS-CoV-2 positive status of samples tested positive for Sarbecovirus with Kylt® SARS-CoV-2 Screening
Kylt® RNA / DNA Purification	31314 / 31315	250 / 50	Combined RNA and DNA purification from all relevant sample matrices
Kylt® RNA / DNA Purification HTP	31826	4x96	Combined, magnetic beads-based purification of RNA and DNA from all relevant sample matrices, suitable for automated high throughput processing
Kylt® Purifier	31436	--	Purification system for magnetic beads. Up to 96 samples in under 45 minutes.

I. 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 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.

Kylt® is a registered trademark.

For *in vitro* use only. Regulatory requirements vary by country, not all of the products described herein may be available in your geographic area.

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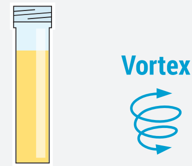
RT-qPCR.SARS-CoV-2.Complete.02, Rev003, November 2020

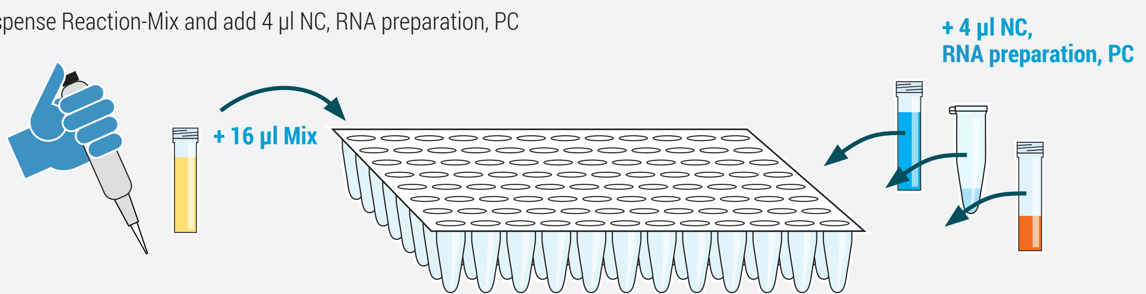
PROTOCOL AT A GLANCE

Real-Time RT-PCR Setup

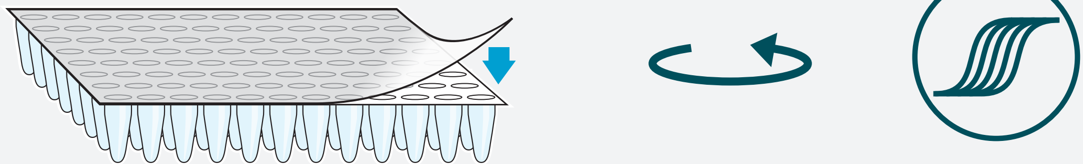
1 Pulse-vortex and spin down



2 Dispense Reaction-Mix and add 4 µl NC, RNA preparation, PC



3 Seal cavities, spin down (recommended), and start cycler



4 Analysis