SIGMA-ALDRICH®

SCAN BEC

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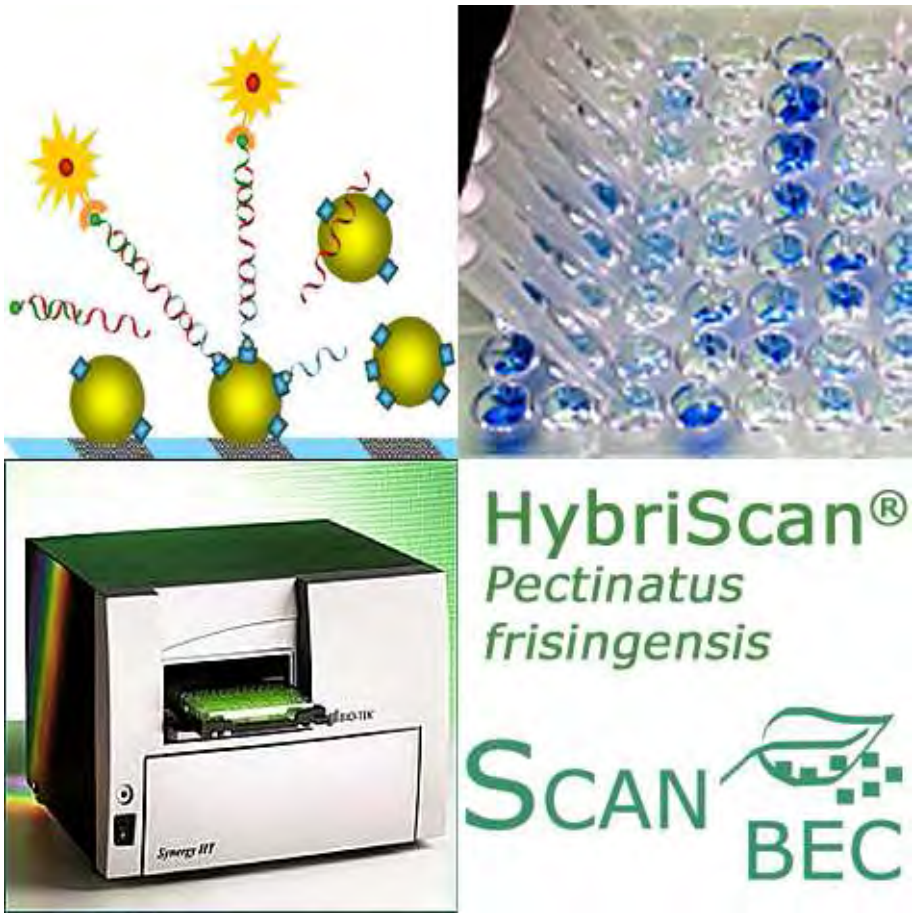
HybriScan®*I*
Pectinatus frisingensis

The rapid and innovative test system for the identification of *Pectinatus frisingensis*

Product-No.: 73582



Deutsche Version der Anleitung siehe unter www.sigma-aldrich.com/hybriscan

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Product Specifications

Cat. No.:	73582
Number of tests:	48 tests
Storage:	4 – 8°C, 12 month
Test duration:	approx. 1 hour
Sensitivity:	1000 CfU/assay
Specificity:	*Pectinatus frisingensis*

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HybriScan®*I Pectinatus frisingensis* –Test Protocol

Working Principle

HybriScan®***I*** *Pectinatus frisingensis* is an enzyme-linked, molecular test system for the detection and identification of *Pectinatus frisingensis* . The HybriScan®***I*** tests are based on the detection of target molecules from the micro-organism of interest by means of specific capture and detection probes in a so called sandwich hybridization. The target molecules of these microbes contained in the sample are captured in a specific microtiter binding plate. All other unbound sample components are removed by several washing steps. In addition to the capture probe, a detection probe is coupled to the target molecule. An enzyme is attached afterwards in a subsequent incubation step. After several washing steps, reaction with a colour substrate gives a blue colouration, which changes to yellow after the addition of a stop solution. The yellow colour enables highly sensitive photometric measurement at 450 nm. Comparison is made with the standard solutions contained in the test kit.

Technical Notes

After starting the test procedure, perform the following steps without interruptions and within the given time limit.

For each sample use single-use pipette tip to avoid cross-contamination.

Close bottles immediately after use and store them at the temperatures specified on the label. Do not interchange caps and bottles.

Samples and standards should be tested together for more accurate results.

Do not mix or replace components from test kits of different charges.

Incubation at room temperature refers to a laboratory temperature of 20 to 25°C.

Do not use the test kit after the expiration date listed on the package.

Safety

All reagents contained in the test kit are for *in vitro* use only.

Test solution D contains formamide. Avoid contact with eyes, skin and the respiratory system. In event of contact with eyes or skin, rinse immediately with plenty of water. If the reagent is inhaled, immediately remove the individual to fresh air and seek medical attention. Stop solution H contains 1 N sulfuric acid. Avoid contact with eyes and skin. In the event of contact with eyes and skin rinse immediately with plenty of water.

Handling of the kit components and disposal of waste should be performed according to standard laboratory safety guidelines.

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Reagents and Storage Conditions

The reagents contained in the test kit are sufficient for at least 48 tests. The kit components should be stored between +4 to +8°C as indicated on the labels. Do not freeze the test kit components!

Kit components:

1. Binding plate, ready to use, 96 wells	1
2. Negative Control [a)] (white screw caps), ready to use	0.2 mL
3. Lysis Reagent A (red screw cap), ready to use	1.0 mL
4. Lysis Buffer B [a)] (red cap), ready to use	4.5 mL
5. Lysis Buffer C [a)] (red cap), ready to use	5.5 mL
6. Test Solution D1 and **D2** (yellow cap), ready to use	5.0 mL
7. Washing Solution E [b)] (blue cap), ready to use	90 mL
8. Enzyme Solution F (green screw cap), dilute a suitable amount 1:100 with washing solution E before usage	0.140 mL
9. Substrate Solution G [b)] (green cap), ready to use	10 mL
10. Stop Solution H (green cap) 1 N sulfuric acid, ready to use	5 mL
11. Glass beads (colourless cap), sterile, ready to use	4 mL

[a)] Components contain SDS, which precipitates at lower temperatures. Equilibrate to room temperature before use.
[b)] Equilibrate to room temperature before use.

Additional equipment and materials (required, not supplied with kit)

- Centrifuge for microreaction tubes (1.5 and 2 mL), 13,000 rpm
- Thermoshaker for microreaction tubes and microwell plate
- 3 Pipettes (2–20 µL, 20–200 µL, 200-1000 µL) with corresponding tips; optional 8-channel pipette (20-200 µL)
- Microwell plate-photometer
- Microreaction tubes (2 mL)

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Test protocol

(1) Sample preparation

Choose and transfer a single bacterial colony from an agar plate into a 2 mL microreaction tube prepared with a spatula tip of glass beads, 40 µL of **Lysis Buffer B** (bottle with red cap) and 10 µL of **Lysis Reagent A*** (microreaction tube with red screw cap). Resuspend bacteria in this lysis solution.

(2) Cell lysis

Incubate samples for 8 minutes at 37°C in a thermoshaker. Add 50 µL of **Lysis Buffer C** (bottle with red cap). Incubate for 8 minutes at 37°C with shaking at 1,400 rpm in the thermoshaker. Centrifuge the samples for 5 minutes at 13,000 rpm. Use 10 µL of this supernatant in protocol step 3 (hybridization).

***Note:** In the case of a large number of samples prepare a Master Mix of **Lysis Reagent A** and **Lysis Buffer B** before use. Pipette 50 µL of the Master Mix to each cell pellet.

Preparation for subsequent steps:

Change the top of the thermoshaker and fix the manifold for microwell plates. Set the temperature to 50°C and shaking speed to 500 rpm. Pipette 45 µL of **Test Solution D1** (bottle with yellow cap) for the Negative Control and for each sample in a separate well of the binding plate. Additionally, pipette 45 µL of **Test Solution D2** for the Negative Control and for each sample in a separate well of the binding plate**.** Cover the plate with a lid and pre-incubate it at 50°C for a minimum of 5 minutes in the thermoshaker.

(3) Hybridization and immobilisation

Add 10 µL of the **Negative Control** to the well filled with **Test Solution D1** and 10 µL into the well filled with **Test Solution D2**.

Add 10 µL of the sample (supernatant from step 2) to the respective well filled with **Test Solution D1** and additional 10 µL of the same sample to the respective well filled with **Test Solution D2**. Afterwards cover the plate with a lid and incubate it in the thermoshaker for 15 minutes at 50°C and 500 rpm.

Note:

Unused stripes of the plate should be stored in the sealed original packing at 4 to 8 °C.

For step 3 only, 2 x 10 µL of the supernatant from step 2 is needed per sample. If further measurements are required, the complete supernatant should be transferred into a new, sterile 1.5 mL microreaction tube and stored at -20°C.

Preparation for subsequent steps:

Dilute a suitable amount of **Enzyme Solution F** (microreaction tube with green screw cap) 1:100 with **Washing Solution E** (bottle with blue cap). Prepare only the amount needed for the test, e. g. for 16 reactions combine 1700 µL **Washing Solution E** and 17 µL **Enzyme Solution F**.

Note:

Briefly spin down Enzyme Solution F prior use to collect the liquid at the bottom of the tube.

The dilution of Enzyme Solution F with Washing Solution E must be prepared just before use and cannot be stored for further tes

(4) Enzymatic reaction

Discard the liquid from each well by inverting and gently beating of the plate. Set the temperature to 25°C. Add 200 µL **Washing Solution E** (bottle with blue cap) and incubate for 2 minutes at room temperature on your bench. Discard the liquid and pipette 100 µL of the diluted Enzyme Solution, prepared as described above "preparation for subsequent steps", into each well. Afterwards the binding plate is covered with a lid and incubated in the thermoshaker for 10 minutes at 25°C and 500 rpm.

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(5) Washing

Discard the liquid from each well. Add 200 µL of **Washing Solution E** (bottle with blue cap) to each well and incubate the microplate (with lid) for 1 minute at 25°C and 500 rpm in the thermoshaker. Repeat washing each well once.

Preparation for subsequent steps:

Switch on the computer and the microplate reader.

(6) Substrate Reaction

After discarding the washing solution from the second wash step, add 100 µL of **Substrate Solution G** (bottle with green cap) to each well. Cover the microplate with a lid and incubate it in a thermoshaker at 25°C and 500 rpm. After a few minutes a blue colouration in contaminated samples is visible. After 2-15 minutes (blue colour of Test Solution D1 is clearly visible, independent of the intensity of the colour of Test Solution D2) all reactions can be stopped by adding 50 µL of **Stop Solution H** (bottle with green cap) to each well. The addition of acid creates a yellow colour change. Mix briefly (10 sec, 500 rpm) in the thermoshaker and remove air bubbles, if present.

(7) Signal read-out using VIS-photometer

Start the reader and open the photometer Software. Insert the microwell plate into the reader, with position A1 rear left. Start the measurement. The instrument measures the absorbance of any position at 450 nm.

(8) Data interpretation

Both signals of the analysed sample will be compared. The ratio of Test Solution D1 to Test Solution D2 signals will be below 2.0 for positive samples.
For example: Value Test Solution D1: 1.045/Test Solution D2: 0.782 results in 1.33. The colony was therefore positive for *Pectinatus frisingensis*.
Bacteria other than *P. frisingensis* generate a high signal for total number of cells (Test Solution D1) but a very low signal for *P. frisingensis* (Test Solution D2) resulting in a signal ratio greater than 2.0. For the measurement to be valid values of Test Solution D1 must be greater than 0.2 and the value of the negative control must be less than 0.1.

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Short Protocol

1. Transfer and resuspend a single colony in a 2 mL microreaction tube prepared with a spatula tip of glass beads, 40 µL of **Lysis Buffer B** (red cap) and 10 µL of **Lysis Reagent A** (red cap); incubate for 8 min at 37 °C in a thermoshaker
2. Add 50 µL of **Lysis Buffer C** (red cap) and incubate for 8 min at 37°C and 1,400 rpm in the thermoshaker
3. Centrifuge for 5 min at 13,000 rpm
4. Pipette 45 µL of **Test Solution D1** and 45 µL of **Test Solution D2** (yellow cap) per each sample (including the negative control) into the wells of the binding plate and pre-incubate for 5 min at 50°C and 500 rpm in the thermoshaker
5. Add 10 µL of the supernatant from step 3 to each well; cover the microwell plate with a lid and incubate for 15 min at 50°C and 500 rpm in the thermoshaker
6. Discard all liquid and wash the plate with 200 µL **Washing Solution E** (blue cap), discard Washing Solution
7. Dilute a suitable amount of **Enzyme Solution F** (green screw cap) 1:100 with **Washing Solution E** (blue cap) and add 100 µL of the mixture to each well of the microplate; cover the plate with a lid and incubate for 10 min at 25°C and 500 rpm in the thermoshaker
8. Discard all liquid and add 200 µL of **Washing Solution E** (blue cap) to each well and incubate for 1 min at room temperature and 500 rpm in the thermoshaker; repeat the washing step once
9. Discard all liquid and add 100 µL **Substrate Solution G** (green cap) per sample to the wells of the microplate; cover the plate with a lid and incubate for 2-15 min at 25°C and 500 rpm in the thermoshaker
10. Add 50 µL **Stop Solution H** (green cap) to each well
11. Place the microplate in a microplate reader and measure the optical density in each well at 450 nm; perform data analysis

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Overview of the HybriScan®*I Pectinatus frisingensis* procedure:

1.Sample preparation
(enrichment, plating)

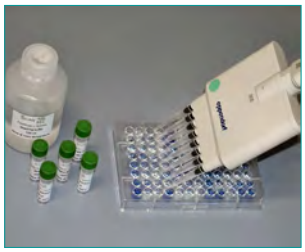
2. Cell lysis

3. Hybridization and Immobilisation
(15 min)

4. Washing
(2 min)

5. Enzyme coupling
(Coupling of enzyme to "sandwich complexes", 10 min)

6. Washing
(removal of unbound components, 2x1 min)



7. Colour reaction
(2-15 min)

8. Signal read out /Test analysis

Advantages

- Rapid, sensitive, reliable
- Easy to handle
- Minimized sample preparation procedure
- High sample throughput using 96 well microplates
- Detects only living organisms