SIGMA-ALDRICH®

SCAN BEC

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HybriScan®*D* Drinks

Rapid test system for qualitative detection of bacteria and yeast in non-alcoholic drinks

Product-No.: 68301

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

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Technical Service:
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Product Specifications

Cat. No.:	68301
Number of tests:	96 tests, incl. standard series
Storage:	4 – 8°C, 12 month
Test duration:	approx. 2-2.5 hours (after pre-enrichment)
Sensitivity:	1 -10 CFU/L (after pre-enrichment)
Specificity:	amongst others yeast of the genera *Saccharomyces*, *Zygosacchromyces*, *Brettanomyces*, *Torulaspora*, *Pichia*, *Candida* and bacteria of the genera *Lactobacillus*, *Acetobacteraceae* and *Alicyclobacillus*

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HybriScan®*D* Drinks–Test Protocol

Working Principle

HybriScan®***D*** Drinks is a rapid molecular test system for the detection of all relevant yeast and bacteria in only one test. HybriScan®***D*** Drinks is based on the detection of target molecules from the microorganism of interest by means of specific capture and detection probes in a so-called sandwich hybridization. The target molecules of the beverage-spoiling contaminants contained in the sample are captured in a specific microtiter binding plate. All other unbound sample components are removed by several washing steps, so that only beer-spoiling microbes are detected in a highly specific way. 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 each of the following steps without interruption and within the given time limits:
For each sample use an individual single-use pipette tip to avoid cross-contamination.
Close bottles immediately after use and store them at the temperatures specified on the labels. 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 96 tests, including 6 standard series. The kit components should be stored between +2 to +8°C as indicated on the labels. Do not freeze the test kit components!

Kit components:

1. Microwell plate, ready to use, 96 wells	1
2. Binding plate, ready to use, 96 wells	1
3. Standards 1 & 2 [a)] (white screw caps); standard 1: blank and standard 2: positive control	0.2 mL each
4. Lysis Reagent A (red screw cap), ready to use	1.2 mL
5. Lysis Buffer B [a)] (red cap), ready to use	4.5 mL
6. Lysis Buffer C [a)] (red cap), ready to use	5.5 mL
7. Test Solution D (yellow cap), ready to use	4.5 mL
8. Washing Solution E [b)] (blue cap), ready to use	90 mL
9. Enzyme Solution F (green screw cap), dilute a suitable amount 1:100 with Washing Solution E before use	0.120 mL
10. Substrate Solution G [b)] (green cap), ready to use	10 mL
11. Stop Solution H (green cap) 1 N sulfuric acid, ready to use	5 mL
12. 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)
- Thermoshaker for microreaction tubes and microwell plates
- Vacuum filtration unit
- 3 Pipettes (2–20 µL, 20–200 µL, 200-1000 µL) with corresponding tips; optional 8-channel pipette (20-200 µL)
- Microwell plate-photometer
- Enrichment medium, incubator
- Microreaction tubes (2 mL), cultivation tubes (12 mL), reagent-reservoirs, membrane filter discs (0.45 µm)

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Test protocol

(1) Sample preparation

Transfer a 2 mL aliquot from the pre-cultivation tube via pipette to a 2 mL microreaction tube that contains a spatula-tip amount of glass beads. Centrifuge the samples for 2 minutes at maximum speed of 13,000 rpm. Remove the supernatant carefully with a pipette.

Note:

Avoid strong shaking after centrifugation to avoid resuspending the bacteria pellet. Centrifuge a second time if necessary. Samples with high yeast content should be centrifuged for 1 minute at 1,000 rpm to allow the yeast to settle. Take 1 mL of the supernatant and proceed with step 1. To increase the sensitivity, start with 2 x 2 mL sample and combine the supernatants.

(2) Cell lysis

Add 40 µL of **Lysis Buffer B** (bottle with red cap) to the cell pellet and 10 µL of **Lysis Reagent A*** (microreaction tube with red screw cap), mix well and incubate for 15 minutes at 37°C in a thermoshaker. Next, add 50 µL of **Lysis Buffer C** (bottle with red cap). Incubate again for 15 minutes at 37°C with shaking at 1,400 rpm in the thermoshaker. Centrifuge the samples for 10 minutes at 13,000 rpm using a microcentrifuge. 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. For quantitative analysis of beverage-spoiling microorganisms we recommend a repeat determination of the 2 standard solutions (microreaction tubes with white screw cap).

To each well of standards (repeat determinations, e.g. A1-H1) and samples add 45 µL of Test Solution D (bottle with yellow cap). Incubate the plate at 50°C for 5 minutes in the thermoshaker.

(3) Hybridization

Add 10 µL of **Standard 1** into the wells A1 and B1; 10 µL of **Standard 2** into C1 and D1. Apply 10 µL of each sample (supernatant from step 2) into the respective well position. Cover the microwell plate with a lid and incubate it in the thermoshaker for 10 minutes at 50°C and 500 rpm.

Note:

When adding the standards and samples, to avoid cooling do not remove the microwell plate from the thermoshaker.

The supernatant from step 2 can be stored at -20°C for future use.

(4) Coupling to the binding plate

Transfer 50 µL of the reaction mixes from each well to the corresponding wells of the binding plate and shake for 10 minutes at 50°C and 500 rpm in the thermoshaker.

Note:

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

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Preparation for subsequent steps:

The **Enzyme Solution F-Washing Solution E** 1:100 dilution must be prepared immediately before use. It cannot be stored. 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.

(5) Enzymatic reaction

Discard the liquid from each well by inverting and gently tapping of the plate on an absorbent layer. Set the temperature to 25°C. Add 200µL **Washing Solution E** (bottle with blue cap) and incubate for 2 minutes at room temperature. Discard the liquid. Pipette 100 µL of the diluted **Enzyme Solution**, prepared as described above ”preparation for subsequent steps”, to each well. Cover the binding plate with a lid and incubate it in the thermoshaker for 10 minutes at 25°C and 500 rpm.

(6) 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 computer and the microplate reader.

(7) 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 for 10 minutes at 25°C and 500 rpm. Stop the reaction 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 shortly (10 sec, 500 rpm) in the thermoshaker and remove air bubbles, if present.

.

Note:

For qualitative analysis results can be measured by visual inspection. Compared to the blanks (A1, B1), which should be colourless, a blue colour change indicates contamination of the sample.

(8) 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.

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(9) Data analysis

For the measurement to be valid, the quotient of the mean value of the positive control (S2) divided by the mean value of the negative control (S1) must be greater than 4.0.

Evaluation of the samples is performed using the following formula:

$$\text{Sample OD\%} = \frac{OD_{Sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}} \times 72.1 OD\%$$

MV mean value
PC positive control (S2)
NC negative control (S1)

Sample OD% values are used to evaluate the sample status:

Samples with OD% values under 10 are considered negative.
Samples with OD% values from 10 to < 20 are considered questionable.
Samples with OD% values ≥ 20 are considered positive.

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Short Protocol

1. Filter 100–1,000 mL of sample (vacuum filtration unit; membrane filter disc; 0.45 µm pore size)
2. After filtration incubate the filter disc in 5 mL of enrichment medium
3. Remove 2 mL of sample from the enrichment medium, add glass beads, centrifuge (13,000 rpm, 2 minutes) and discard the supernatant
4. Add 40 µL of **Lysis Buffer B** (red cap) to the pellet and add 10 µL of **Lysis Reagent A** (red screw cap); mix and incubate for 15 minutes at 37°C in a thermoshaker
5. Add 50 µL of **Lysis Buffer C** (red cap) and incubate for 15 minutes at 37°C and 1,400 rpm in the thermoshaker
6. Centrifuge for 10 min at 13,000 rpm
7. Pipette 45 µL of **Test Solution D** (yellow cap) per sample (including the standards) into the wells of a microplate and pre-incubate for at least 5 minutes at 50°C and 500 rpm in the thermoshaker
8. Add 10 µL of the supernatant from step 6 to each well (row A1–H1 is reserved for the respective standards); cover the microwell plate with a lid and incubate for 10 min at 50°C and 500 rpm in the thermoshaker
9. Transfer 50 µL of reaction mixes to the binding plate and incubate for 10 min at 50°C and 500 rpm in a thermoshaker
10. Discard all liquid and wash the plate with 200 µL **Washing Solution E** (blue cap), discard Washing Solution
11. 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 minutes at 25°C and 500 rpm in the thermoshaker
12. Discard all liquid and add 200 µL of **Washing Solution E** (blue cap) to each well and incubate for 1 minute at room temperature and 500 rpm in the thermoshaker; repeat the washing step once
13. 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 5-15 minutes at 25°C and 500 rpm in the thermoshaker
14. Add 50 µL **Stop Solution H** (green cap) to each well
15. 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®*D* Drinks procedure:

1.Sample filtration
(100- 1000 ml water,
15 min)

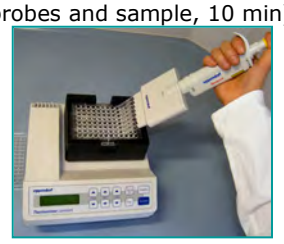
2.Enrichment culture, optional

3. Centrifugation/Cell-Lysis
(2 ml sample, 13.000 rpm;
37°C, 45-60 min)

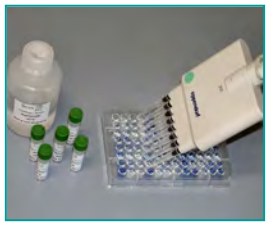
4. Hybridisation
(Forming of "sandwich complexes" between specific probes and sample, 10 min)

5. Immobilisation
(Binding of the „sandwiches" to an affinity plate, 10 min)

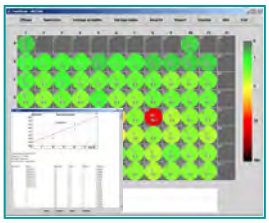
6. Enzym coupling
(Coupling of an enzyme to the „sandwiches", 10 min)



7. Washing
(Removal of unbound components, 2x1 min)



8. Encymatic reaction
(Enzymatic colour reaction,
5-15 min)



9. Signal read out/Test analysis
(450 nm)

Advantages

- Rapid, sensitive, reliable
- Specific for living cells
- Time saving of 2 to 4 days in comparison to cultivation based assays
- Easy to handle
- Minimal sample preparation
- High sample throughput using 96-well microplates