SIGMA-ALDRICH®

SCAN BEC

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HybriScan®*D Listeria*

Rapid test system for the detection of *Listeria spp.*

Product-No.: 55661

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

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Product Specifications

Cat. No.:	55661
Number of tests:	96 tests, incl. standard series
Storage:	4 – 8°C, 12 month
Test duration:	approx. 2-2.5 hours (after pre-enrichment)
Pre-enrichment time:	48 hours
Sensitivity:	1 CFU/25 g (after pre-enrichment)
Specificity:	*Listeria spp.*

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HybriScan®*D Listeria*–Test Protocol

Working Principle

HybriScan®***D*** *Listeria* is a rapid molecular test system for the qualitative detection of the genus *Listeria*. The HybriScan® technology 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 microorganisms contained in the sample are captured in a specific microwell binding plate with the help of the capture probe. 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 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 – 4 [a] (white screw caps); blank and different concentrations of synthetic RNA as positive control and for preparation of a calibration curve for quantitative measurements	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	4.5mL
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
- 3 Pipettes (2–20 µL, 20–200 µL, 200-1000 µL) with corresponding tips
- Microwell plate-photometer
- Enrichment medium [half-fraser - and fraser – boullion] and incubator
- Microreaction tubes (2 mL), cultivation tubes (12 mL)
- Stomacher bag with a filter, Stomacher

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Test protocol

(1) Sample preparation

25 g of the test specimen are weighed in a sterile Stomacher bag (with filter), 225 mL half-fraser-bouillon are added and the mixture is homogenized for 1 minute in a Stomacher. Afterwards samples are incubated for 24 hours at 30°C. After 24 hours of incubation 0.1 mL of the pre-enrichment culture are transferred to 10 mL of fraser-bouillon and is conducted for 24 hours at 37°C. After 24 hours of incubation transfer a 2 mL aliquot of the main enrichment culture 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.

(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 of 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. 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; 10 µL of **Standard 3** into E1 and F1 and 10 µL of **Standard 4** into G1 and H1. 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.

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:

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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”, into 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 the 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.

Data analysis using the HybriScan®-Software

(8) Signal read-out and data analysis using the HybriScan®-Software

Start the reader and open the HybriScan®-Software. Insert the microwell plate into the reader, with position A1 rear left. Click button **Start Measurement** and choose the appropriate test. Click **Start**. The instrument measures the absorbance of any well. Results are saved automatically in the database. Alternatively, results of tests saved as text file can be opened by choosing option **File/Open** in the main menu.
The graphical layout of the results corresponds to the position of the sample in the microwell plate. By clicking on each position, you can enter the name of the sample. In the lower pane of the graphical user interface the user name and other comments can be inserted. For each position you can hide or unhide parameters by choosing the button **View.**
Click the button **Report**. In the upper pane of the window the regression line calculated from the four standards is displayed. Values of standards are represented by red dots. They should be close to the regression curve (blue line). Your results can be printed by choosing the option **Print Report.** For post processing (like in Microsoft Excel) results can be exported by choosing the option **Export Report**.

(9) Data interpretation

Based on the absorbance measurement, HybriScan®-Software will help to evaluate the analyzed samples. The quantitative analysis is performed automatically on the basis of the standard values (A1 to H1). For each well the corresponding number of cells are calculated and indicated by the software numerically and with a colour code. The change of green to red enables a fast visual recognition of contaminated samples.

The cell number calculated by the HybriScan®-Software reflects the amount of cell equivalents in the 10 µL sample introduced to the test solution. If you started with 2mL culture, you have to multiply the calculated cell number by 5 to get the amount of cells per mL.

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Furthermore, the mean values of optical density of the negative control (standard 1) are used to evaluate, if a sample is positive, questionable or negative.

The HybriScan™-Software enables both, graphical and tabular representation of the measurement data of the respective samples. Optical density and calculated numbers of cells are displayed by the software tool.

Data analysis without the HybriScan®-Software

(10) 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.

(11) Internal control

We recommend using all four standards as internal controls for the test procedure. If the analysis is performed correctly your measured data will be near the regression line. We recommend this method especially for unexperienced user.

For linear regression set standard1 = 0, standard2 = 1, standard3 = 3 and standard4 = 10 at the abscissa (x-coordinate). The straight line must subtend the y-axis at S1 =0.

Regression line offers only verification of analysis-quality; a quantification is not possible.

For experienced user it is sufficient to use only standard S1 and S3. These standards are necessary for a qualitative data analysis.

(12) Qualitative analysis

For the measurement to be valid, the quotient of the mean value of the positive control (S3) 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:

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

MV mean value
PC positive control (S3)
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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(13) Quantitative analysis

For quantitative analysis (semi - quantitative analysis) use following formula to calculate cell count of your samples:

$$\text{Cell count}_{\text{Sample}} = \frac{\text{OD}_{\text{Sample}} \times \text{Cell count}_{\text{Standard}}}{\text{MV OD}_{\text{Standard}}}$$

MV mean value

For calculating the cell count, choose a standard, which has a OD next to sample – OD.

Cell count – standards:

S1 = 0

S2 = 1000

S3 = 3000

S4 = 10000

Note:

Quantitative analysis is only possible, if sensitivity was reached (see product specifications), your measured data are near the regression line and no pre-enrichment was performed!

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Short Protocol

1. Place 25 g of the sample in a sterile Stomacher bag with filter
2. Add 225 mL half-fraser-boullion and homogenize for 1 minute in a Stomacher (first pre-enrichment culture)
3. Incubate 24 h at 30°C
4. Transfer 0.1 mL first pre-enrichment culture to 10 mL fraser-boullion
5. Incubate 24 h at 37 °C
6. transfer a 2 mL aliquot of the main enrichment culture to a 2 mL microreaction tube, add one spatula of glass beads, centrifuge (13,000 rpm, 2 minutes) and discard the supernatant
7. Add 40 µL of **Lysis Buffer B** (red cap) to the pellet; add 10 µL of **Lysis Reagent A** (red screw cap); mix and incubate for 15 minutes at 37°C in a thermoshaker
8. Add 50 µL of **Lysis Buffer C** (red cap) and incubate for 15 minutes at 37°C and 1,400 rpm in the thermoshaker
9. Centrifuge for 10 minutes at 13,000 rpm
10. Pipette 45 µL of **Test Solution D** (yellow cap) per sample (including the standards) into the wells of a microplate and pre-incubate for 5 minutes at 50°C and 500 rpm in the thermoshaker
11. Add 10 µL of the supernatant from step 9 to each well (row A1–H1 is reserved for the respective standards); cover the microwell plate with a lid and incubate for 10 minutes at 50°C and 500 rpm in the thermoshaker
12. Tranfer 50 µL of reaction mixes to the binding plate and incubate for 10 minutes at 50°C and 500 rpm in a thermoshaker
13. Discard all liquid and wash the plate with 200 µL **Washing Solution E** (blue cap), discard Washing Solution
14. 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 binding plate; cover the plate with a lid and incubate for 10 minutes at 25°C and 500 rpm in the thermoshaker
15. 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
16. Discard all liquid and add 100 µL **Substrate Solution G** (green cap) per sample to the wells of the binding plate; cover the plate with a lid and incubate for 10 minutes at 25°C and 500 rpm in the thermoshaker
17. Add 50 µL **Stop Solution H** (green cap) to each well
18. Place the Plate 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 Listeria spp.* procedure:

1. Sample preparation
(optional enrichment)

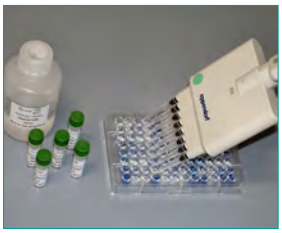
2. Cell lysis
(2 mL Sample, 13.000 rpm; 37°C, 45-60 min)

3. HybriScan® - test solution
(forming of „sandwich complexes" between specific probes and the sample, 10min)

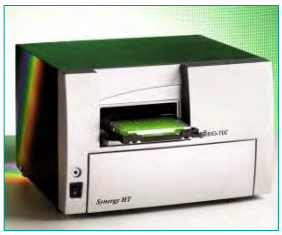
4. Immobilisation
(Binding of the „sandwiches" to the binding plate, 10 min)

5. Enzyme reaction
(Coupling of an enzyme to the „sandwiches", 10 min)

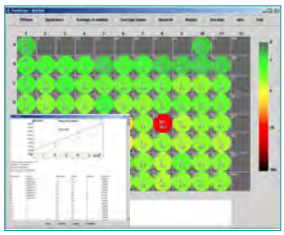
6. Washing
(Removal of unbound components, 2x 1 min)



7. Color reaction
(10 min)



8. Signal read out



9. Test analysis

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