

HybriScan® I

Lactobacillus brevis

The rapid and innovative test system for the identification of *Lactobacillus brevis*

Product-No.: 75724



Contact information:

HybriScan® - Rapid Test System (R&D)

Dr. Helmut Maucher
 Phone: (+49) – 3494 – 6364 15
 e-mail: contact@scanbec.de

Sales Organisations

Argentina

SIGMA-ALDRICH DE
 ARGENTINA S.A.
 Free Tel: 0810 888 7446
 Tel: (+54) 11 4556 1472
 Fax: (+54) 11 4552 1698

Australia

SIGMA-ALDRICH PTY LTD.
 Free Tel: 1800 800 097
 Free Fax: 1800 800 096
 Tel: (+61) 2 9841 0555
 Fax: (+61) 2 9841 0500

Austria

SIGMA-ALDRICH HANDELS
 GmbH
 Tel: (+43) 1 605 81 10
 Fax: (+43) 1 605 81 20

Belgium

SIGMA-ALDRICH NV/SA.
 Free Tel: 0800 14747
 Free Fax: 0800 14745
 Tel: (+32) 3 899 13 01
 Fax: (+32) 3 899 13 11

Brazil

SIGMA-ALDRICH BRASIL LTDA.
 Free Tel: 0800 701 7425
 Tel: (+55) 11 3732 3100
 Fax: (+55) 11 5522 9895

Canada

SIGMA-ALDRICH CANADA LTD.
 Free Tel: 1800 565 1400
 Free Fax: 1800 265 3858
 Tel: (+1) 905 829 9500
 Fax: (+1) 905 829 9292

China

SIGMA-ALDRICH (SHANGHAI)
 TRADING CO. LTD.
 Free Tel: 800 819 3336
 Tel: (+86) 21 6141 5566
 Fax: (+86) 21 6141 5567

Czech Republic

SIGMA-ALDRICH S.R.O.
 Tel: (+420) 246 003 200
 Fax: (+420) 246 003 291

Denmark

SIGMA-ALDRICH DENMARK
 A/S
 Tel: (+45) 43 56 59 10
 Fax: (+45) 43 56 59 05

Finland

SIGMA-ALDRICH FINLAND OY
 Tel: (+358) 9 350 9250
 Fax: (+358) 9 350 92555

France

SIGMA-ALDRICH CHIMIE S.à.r.l.
 Free Tel: 0800 211 408
 Free Fax: 0800 031 052
 Tel: (+33) 474 82 28 00
 Fax: (+33) 474 95 68 08

Germany

SIGMA-ALDRICH CHEMIE
 GmbH
 Free Tel: 0800 51 55 000
 Free Fax: 0800 64 90 000
 Tel: (+49) 89 6513 0
 Fax: (+49) 89 6513 1160

Greece

SIGMA-ALDRICH (O.M.) LTD.
 Tel: (+30) 210 994 8010
 Fax: (+30) 210 994 3831

Hungary

SIGMA-ALDRICH Kft
 Ingyenes zöld telefon: 06 80 355
 355
 Ingyenes zöld fax: 06 80 344 344
 Tel: (+36) 1 235 9055
 Fax: (+36) 1 235 9050

India

SIGMA-ALDRICH CHEMICALS
 PRIVATE LIMITED
 Telephone
 Bangalore: (+91) 80 6621 9600
 New Delhi: (+91) 11 4165 4255
 Mumbai: (+91) 22 2570 2364
 Hyderabad: (+91) 40 6684 5488
 Fax
 Bangalore: (+91) 80 6621 9650
 New Delhi: (+91) 11 4165 4266
 Mumbai: (+91) 22 2579 7589
 Hyderabad: (+91) 40 6684 5466

Ireland

SIGMA-ALDRICH IRELAND
 LTD.
 Free Tel: 1800 200 888
 Free Fax: 1800 600 222
 Tel: (+353) 1 404 1900
 Fax: (+353) 1 404 1910

Israel

SIGMA-ALDRICH ISRAEL LTD.
 Free Tel: 1 800 70 2222
 Tel: (+972) 8 948 4100
 Fax: (+972) 8 948 4200

Italy

SIGMA-ALDRICH S.r.l.
 Numero Verde: 800 827018
 Tel: (+39) 02 3341 7310
 Fax: (+39) 02 3801 0737

Japan

SIGMA-ALDRICH JAPAN K.K.
 Tokyo Tel: (+81) 3 5796 7300
 Tokyo Fax: (+81) 3 5796 7315

Korea

SIGMA-ALDRICH KOREA
 Free Tel: (+82) 80 023 7111
 Free Fax: (+82) 80 023 8111
 Tel: (+82) 31 329 9000
 Fax: (+82) 31 329 9090

Malaysia

SIGMA-ALDRICH (M) SDN. BHD
 Tel: (+60) 3 5635 3321
 Fax: (+60) 3 5635 4116

Mexico

SIGMA-ALDRICH QUÍMICA,
 S.A. de C.V.
 Free Tel: 01 800 007 5300
 Free Fax: 01 800 712 9920
 Tel: 52 722 276 1600
 Fax: 52 722 276 1601

The Netherlands

SIGMA-ALDRICH CHEMIE BV
 Free Tel: 0800 022 9088
 Free Fax: 0800 022 9089
 Tel: (+31) 78 620 5411
 Fax: (+31) 78 620 5421

New Zealand

SIGMA-ALDRICH NEW
 ZEALAND LTD.
 Free Tel: 0800 936 666
 Free Fax: 0800 937 777
 Tel: (+61) 2 9841 0555
 Fax: (+61) 2 9841 0500

Norway

SIGMA-ALDRICH NORWAY AS
 Tel: (+47) 23 17 60 60
 Fax: (+47) 23 17 60 50

Poland

SIGMA-ALDRICH Sp. z o.o.
 Tel: (+48) 61 829 01 00
 Fax: (+48) 61 829 01 20

Portugal

SIGMA-ALDRICH QUÍMICA,
 S.A.
 Free Tel: 800 202 180
 Free Fax: 800 202 178
 Tel: (+351) 21 924 2555
 Fax: (+351) 21 924 2610

Russia

SIGMA-ALDRICH RUS, LLC
 Tel: +7 (495) 621 6037
 Fax: +7 (495) 621 5923

Singapore

SIGMA-ALDRICH PTE. LTD.
 Tel: (+65) 6779 1200
 Fax: (+65) 6779 1822

South Africa

SIGMA-ALDRICH
 SOUTH AFRICA (PTY) LTD.
 Free Tel: 0800 1100 75
 Free Fax: 0800 1100 79
 Tel: (+27) 11 979 1188
 Fax: (+27) 11 979 1119

Spain

SIGMA-ALDRICH QUÍMICA,
 S.A.
 Free Tel: 900 101 376
 Free Fax: 900 102 028
 Tel: (+34) 91 661 99 77
 Fax: (+34) 91 661 96 42

Sweden

SIGMA-ALDRICH SWEDEN AB
 Tel: (+46) 8 742 4200
 Fax: (+46) 8 742 4243

Switzerland

SIGMA-ALDRICH CHEMIE
 GmbH
 Free Tel: 0800 80 00 80
 Free Fax: 0800 80 00 81
 Tel: (+41) 81 755 2828
 Fax: (+41) 81 755 2815

United Kingdom

SIGMA-ALDRICH COMPANY
 LTD.
 Free Tel: 0800 717 181
 Free Fax: 0800 378 785
 Tel: (+44) 1747 833 000
 Fax: (+44) 1747 833 313
 SAFC (UK) Free Tel: 0800 71 71
 17

United States

SIGMA-ALDRICH
 P.O. Box 14508
 St. Louis, Missouri 63178
 Toll-Free: 800 325 3010
 Toll-Free Fax: 800 325 5052
 Call Collect: (+1) 314 771 5750
 Tel: (+1) 314 771 5765
 Fax: (+1) 314 771 5757

Internet

sigma-aldrich.com

Technical Service:

flukatec@sial.com

Product Specifications

Cat. No.: 75724
Number of tests: 48 tests
Storage: 4 – 8°C, 12 month
Test duration: approx. 1 hour
Sensitivity: 1000 CfU/assay
Specificity: *Lactobacillus brevis*

HybriScan®*I* *Lactobacillus brevis*–Test Protocol

Working Principle

HybriScan®*I* *Lactobacillus brevis* is an enzyme-linked, molecular test system for the detection and identification of *Lactobacillus brevis*. 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.

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)

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.

***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.

(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).

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 tests.

(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 of 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.

(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 *Lactobacillus brevis*.

Bacteria other than *L. brevis* generate a high signal for total number of cells (Test Solution D1) but a very low signal for *L. brevis* (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.

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

Overview of the HybriScan® I *Lactobacillus brevis* 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