RiboFlow *Cronobacter* Detection Kit

Manual, version 2, May 2014

Ordering number **51-416113**

24 Assays

Store at: +2...+25°C

SY-LAB Geräte GmbH
Tullnerbachstrasse 61-65, A-3011 Neupurkersdorf, AUSTRIA
Tel. +43-2231-62252-0
Fax: +43-2231-62193
E-mail: sales@sylab.com
Technical Support: supportbio@sylab.com
www: www.sylab.com

RiboFlow *Cronobacter* Detection Kit

Table of Contents

1. Kit components and storage

Solution A, 2 ml
Solution B, 1 ml
Solution C, 2 ml
RiboFlow *Cronobacter* Lateral Flow Assay device, 24 pcs. (4 × 6)
Reaction tubes, 30 pcs.
Manual

All kit components can be stored at +2..+25°C.

2. Accessory materials and equipment

Necessary:
- Micropipettes
- Sterile pipette tips
- Microcentrifuge (relative centrifugal force at least 2000 × g), e. g. from SY-LAB Geräte GmbH, ordering number 51-410000
- Incubator for RiboFlow procedure: strongly recommended for reliable temperature maintenance during RiboFlow protocol: Small incubator with viewing window, e. g. Mini-Incubator IL10 (SY-LAB Geräte GmbH, ordering number 51-410100)
- Incubator for pre-enrichment / enrichment
- Buffered Peptone Water for pre-enrichment

If impedance analysis is used:
- BacTrac impedance analyser (contact SY-LAB Geräte GmbH for further information)
- Pre-filled BiMedia 145A measuring cells, Vancomycin discs included (SY-LAB Geräte GmbH, ordering number 41-441452), or
- BiMedia 145A base, Vancomycin included (SY-LAB Geräte GmbH, ordering number 41-471456) for filling of
- sterile disposable measuring cells with 2 or 4 electrodes (SY-LAB Geräte GmbH, ordering numbers 41-440002 or 41-440004, respectively)

For use without impedance analysis:
- Sterile (test) tubes, volume 10 ml or more
- BiMedia 145A base, Vancomycin included (SY-LAB Geräte GmbH, ordering number 41-471456)

Optional / recommended:

- Additional sterile 1.5 ml reaction tubes (for confirmation of suspicious single colonies)
- LB medium (for confirmation of suspicious single colonies)
- RiboFlow manipulation plate (SY-LAB Geräte GmbH, ordering number 51-410110)

3. Product use

For the detection of *Cronobacter* spp. from enrichments in BiMedia 145A (without impedance analysis) or BiMedia 145A measuring cells (with impedance analysis), and for confirmation of suspicious single colonies from agar plates. This kit is not approved for use in human diagnostics. During performance of the test protocol, all due care and attention should be exercised in handling kit components (see chapter 4. "Safety information").

4. Safety information

When handling with RiboFlow kits, please refer to the safety data sheets for RiboFlow kit components. These safety data sheets are available on our website (www.sylab.com) for download by registered users. Please observe general safety measures for handling chemicals. Never store kit components together with food. Always wear disposable gloves, protective goggles and suitable protective clothing when working with chemicals.

Caution: *Cronobacter spp. are pathogenic to humans. Follow your national safety regulations for handling of microorganisms pathogenic to humans and take the appropriate measures to prevent infections. Inactivate contaminated material by disinfection and autoclaving.*

5. Product warranty and limitation of warranty

SY-LAB Geräte GmbH guarantees the performance of this product as described in chapter 7 "Specifications/Performance" and for the intended use to the expiration date given on the label. The purchaser must determine the suitability of the product for its particular use and adjust reaction conditions if necessary. SY-LAB Geräte GmbH does not assume responsibility for any consequences or damage whatsoever resulting from use of this product. Should the product fail due to any reason other than misuse or incorrect storage, SY-LAB Geräte GmbH

will replace it free of charge or refund the purchase price after written agreement. We reserve the right to change this product anytime to enhance performance or design. Should there be any technical problems, please do not hesitate to contact us for quick and straightforward help.

6. Quality control

Quality and assay performance of this product are monitored for each lot following Standard Operating Procedures. Quality control certificates are available on our website (www.sylab.com) for download by registered users.

7. Specifications/Performance

An analytical limit of detection of 2.88×10^{10} copies of the target nucleic acid molecule is verified for each lot of RiboFlow *Cronobacter* Detection Kit.

8. Customer service

For technical advice, please contact our customer service (E-mail: supportbio@sylab.com). Our customers are a valuable source of information concerning their special applications and requirements. Your feed-back, information and comments are very helpful for us, since we constantly seek to enhance our products. Please contact us if you have suggestions concerning our products.

9. Introduction

Routinely, the detection of *Cronobacter* spp. is achieved by using classical microbiological and biochemical methods, which are characterized by long duration and high costs. Thus, faster, reliable molecular biology-based methods are in great demand. Impedance methods are now widely distributed and meet the demands of cost-effective and automated screening for *Cronobacter* spp. A rapid method for confirmation of positive screening results based on molecular biology considerably reduces time and costs in addition to enhanced specificity compared to microbiological/biochemical methods. The RiboFlow *Cronobacter* Detection Kit was developed to enable highly specific but at the same time economically priced detection of *Cronobacter* spp. within just a few minutes

after BacTrac screening or after enrichment without impedance analysis, with little hands-on time and without additional costly equipment.

10. Test principle

With RiboFlow *Cronobacter*, a *Cronobacter* spp.-specific ribosomal RNA sequence is detected in a simple lateral flow assay format, using a crude cell extract from an enriched culture. Tedious nucleic acid purification or enzymatic amplification of target sequence is not necessary.

Working steps:

Step	**Duration**
Pre-enrichment	18-24 h
Impedance analysis or enrichment	8-24 h
Sample preparation	ca. 10 min
Lateral flow assay	max. 15 min
Evaluation	

11. Protocol

Important notes:

1. This assay is intended for the analysis of enriched samples (selective enrichment or impedance medium). It can also be used for confirmation of suspicious single colonies from (selective) agar plates.
2. This assay should NOT be carried out right after the detection threshold has been surpassed in BacTrac impedance analysis, but only after a well-defined growth curve has developed. On the other hand, the assay should NOT be carried out using old cultures (well above 24 hours incubation time). In both cases, it cannot be guaranteed that the sample contains enough metabolically active cells and thus enough ribosomal RNA for analysis!
3. For the same reason, it should be avoided to store enriched samples for prolonged time before carrying out the test. Ideally, the assay should be performed right after taking the sample. Only bacterial pellets after centrifugation or bacteria resuspended in Solution A may be stored as pellets or lysates at -20°C for prolonged time (a few weeks).

4. Always agitate enriched cultures gently without spilling, or homogenise by pipetting up and down before taking a sample.
5. The bacterial pellet must be thoroughly and homogenously resuspended after mixing with Solution B!
6. The rest of an enriched sample (measuring cell or enrichment tube) can be stored refrigerated for a few hours until a result is available, to enable a second analysis on the same day, if necessary. It cannot be guaranteed that refrigerated enriched samples give correct results after more than one day of storage, or even later!
7. Centrifugations must be carried out for at least 5 minutes with a relative centrifugal force (RCF) of at least 2000 × g to ensure sedimentation of bacteria. Information regarding the RCF can be found in the user manual of your microcentrifuge.
8. It is possible to analyse more than one sample at the same time, but it is advisable to keep time in-between working steps as short as possible (not more than 1 minute), especially after the incubation step with Solution B.
9. The specificity of a nucleic acid hybridisation assay is strongly dependent on temperature, especially when no washing step is performed, as in a lateral flow assay setting, and when closely related species have to be discriminated. Therefore, it is **absolutely essential to run the RiboFlow *Cronobacter* lateral flow assay at no less than +45°C** and to evaluate it **immediately** after 15 minutes runtime. We recommend using a mini-incubator with viewing window (with the temperature of the incubator set to +48°C), in combination with a pre-warmed (!) RiboFlow manipulation plate (both available at SY-LAB Geräte GmbH), to ensure a reaction temperature in the test device of at least +45°C. Do not keep the devices at ambient temperature for too long before evaluation, because false positive signals may arise after completion of the test and during cooling down.
10. Always work with sterile pipette tips to avoid microbial or nuclease contamination of kit components.

If you have further questions concerning this kit, please contact SY-LAB Geräte GmbH, we are glad to assist you!

A. Pre-enrichment and Enrichment / BacTrac screening

For preparation of BiMedia 145A base + Vancomycin (41-471456, for use in tubes without impedance analysis or for filling of disposable sterile measuring cells for impedance analysis), and for preparation of pre-filled BiMedia 145A measuring cells (41-441452, for impedance analysis), refer to the respective media preparation instructions. For preparation of samples and BacTrac settings/measuring parameters, refer to the BacTrac application sheet(s) for

Cronobacter. These documents are available on our website (www.sylab.com) for download by registered users.

Note: *Vancomycin discs added to pre-filled measuring cells will not dissolve!*

A1) Pre-enrichment:

- Homogenise 100 g of sample in 900 ml of pre-warmed Buffered Peptone Water and incubate at +37° for 18-24 h. Subsequently, carry out enrichment in BiMedia 145A + Vancomycin (without impedance analysis), or perform BacTrac screening in BiMedia 145A + Vancomycin (measuring cells filled by yourself or pre-filled measuring cells).

A2) Enrichment:

a) Enrichment without impedance analysis:

- Pre-warm tubes filled with 9 ml of the prepared BiMedia 145A + Vancomycin to +42°C.
- Transfer 0.1 ml of the homogenised pre-enriched sample to the pre-warmed medium and incubate at +42°C for 18-24 h.
- Subsequently, carry out standard protocol for RiboFlow *Cronobacter* as described in point B (sample preparation and processing is carried out as described in B.2.1).

b) BacTrac screening (impedance analysis):

- Pre-warm measuring cells filled with 9 ml of BiMedia 145A + Vancomycin (filled by yourself, or pre-filled measuring cells) to +42°C.
- Transfer 0.1 ml of a homogenised pre-enriched sample to a pre-warmed measuring cell + Vancomycin.
- Start BacTrac analysis.

Measuring parameters: Temperature: +42°C
Duration: 8-24 h
Interval time: 10 min.
Delay time: 1 h
Evaluation type: M2
Threshold M-value: 5% (E-value not to be considered)

- When the BacTrac analysis is finished, carry out standard protocol for RiboFlow *Cronobacter* as described in point B. Only BacTrac - reactive,

i.e. positive samples, are analysed. Sample preparation and processing is carried out as described in B.2.1 (sample preparation and processing for suspicious single colonies is described in B.2.2).

B. Standard protocol for RiboFlow *Cronobacter*

B.1 Preparations

Pre-warm Solution C and the required number of RiboFlow lateral flow test devices at +45°C for at least 10 minutes before the assay is carried out. A tray or similar (e. g. RiboFlow manipulation plate) is convenient to handle several tests at once. The remaining kit components are used at ambient temperature.

Important note: *Pre-warm RiboFlow manipulation plate in the incubator for at least 1 h before use, to ensure correct RiboFlow reaction temperature during test performance!*

B.2 Sample preparation and processing

Generally, liquid cultures should be gently agitated (do not spill) or homogenised by pipetting up and down before taking samples for this test.

B.2.1 Samples from enrichments / BacTrac screening

- Enrichment in tubes: After enrichment, transfer 0.5 ml of homogenised sample from a tube to a 1.5 ml reaction tube.
  Impedance analysis: After the incubation step in the BacTrac device, transfer 0.5 ml of a BacTrac-reactive (i.e. positive) homogenised sample from the measuring cell to a reaction tube. If duplicates have been analysed, 250 µl each of the respective (positive) duplicates are pooled. Samples / measuring cells not reactive in the BacTrac screening are termed negative and are not tested further.
- Centrifuge bacteria for 5 minutes at a minimum of 2000 × g.
- Carefully remove and discard supernatant without losing the bacterial pellet.
- Resuspend bacterial pellet thoroughly but carefully in 50 µl of Solution A by pipetting up and down, avoid foaming.
- Add 25 µl of Solution B to the sample and mix thoroughly (vortex if possible). Now the bacterial pellet must be completely dissolved. Incubate mixture at ambient temperature for 5 minutes.

Note: *After addition of Solution B, the recommended incubation/reaction conditions must be followed, otherwise erroneous results may occur.*

- Add 60 µl of the pre-warmed (+45°C) Solution C to the sample and mix. Proceed immediately with step B.3 (Lateral Flow Assay).

B.2.2 Single colonies from agar plates

- With a sterile toothpick or inoculation needle, pick a single colony from the plate, transfer it into a sterile 1.5 ml reaction tube containing 0.5 ml of a non-selective medium (e. g. LB medium), and incubate 2-3 hours at +37°C.
- Centrifuge bacteria for 5 minutes at a minimum of 2000 × g.
- Carefully remove and discard supernatant without losing the bacterial pellet.
- Resuspend bacterial pellet thoroughly but carefully in 50 µl of Solution A by pipetting up and down, avoid foaming.
- Add 25 µl of Solution B to the sample and mix thoroughly (vortex if possible). Now the bacterial pellet must be completely dissolved. Incubate mixture at ambient temperature for 5 minutes.

Note: *After addition of Solution B, the recommended incubation/reaction conditions must be followed, otherwise erroneous results may occur.*

- Add 60 µl of the pre-warmed (+45°C) Solution C to the sample and mix. Proceed immediately with step B.3 (Lateral Flow Assay).

B.3 Lateral Flow Assay

Quickly apply the sample to the sample port of a pre-warmed (+45°C) RiboFlow *Cronobacter* lateral flow assay device (Fig. 1) resting horizontally on a flat surface. Let sample enter into the sample application pad. As soon as the pink front of liquid is visible at the bottom of the viewing window (this can take 1-2 minutes, depending on the viscosity of the sample), let the assay run for a maximum of 15 minutes at +45°C, then evaluate the result immediately (step B.4). Handle device carefully after application of sample to avoid spillage.

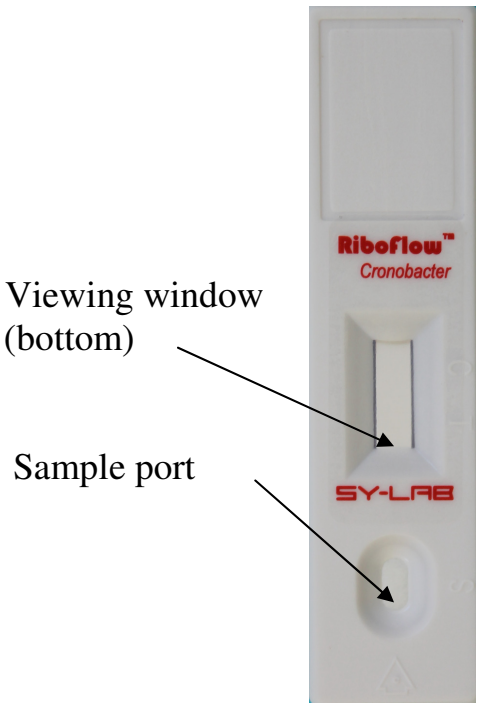


Fig. 1: RiboFlow *Cronobacter* lateral flow assay device

B.4 Evaluation

Note: *The RiboFlow Cronobacter lateral flow assay must be evaluated* ***immediately*** *after 15 minutes runtime at +45°C. Runtimes exceeding 15 minutes might lead to false positive results, especially at temperatures < +45°C. Unspecific bands may appear also a few minutes after the test is finished and is cooling to ambient temperature.*

Evaluation: Figs. A-C are showing viewing windows displaying possible results of RiboFlow *Cronobacter*.

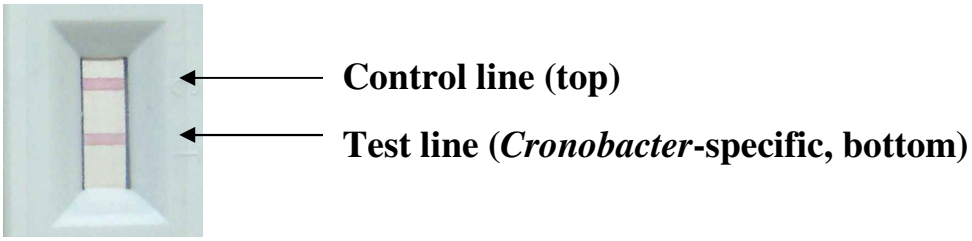
A: Positive for *Cronobacter*: If both control line (top) and *Cronobacter*-specific test line (bottom) are visible in the viewing window, a result is termed positive for *Cronobacter*. Sometimes the control line (top) is very faint in strongly positive samples.

B: Negative result: If only the control line (top) is visible, *Cronobacter* could not be detected with the assay, and the result is termed negative.

C: Invalid result: If no lines are visible at all, some error has occurred. Such a result is invalid, and the test has to be repeated from point B.1, using a new RiboFlow device.

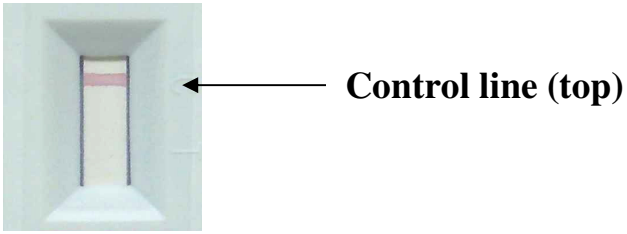
To file a permanent record of results (if desired), we recommend photography using a digital camera immediately at the end of the runtime.

A. Positive result for *Cronobacter*:



Control line (top) and test line (bottom) visible

B. Negative result:



Only control line (top) visible

C. Invalid result:

No line visible

12. Ordering information

RiboFlow *Cronobacter* Detection Kit, 24 assays, ordering number 51-416113
BiMedia 145A, 120 pre-filled measuring cells, Vancomycin-discs included, ordering number 41-441452
BiMedia 145A base, 400 g, Vancomycin included, ordering number 41-471456
Disposable sterile empty measuring cells with 2 electrodes, 400 pcs., ordering number 41-440002 (M-value only) or
Disposable sterile empty measuring cells with 4 electrodes, 400 pcs., ordering number 41-440004 (M- and E-value)
Minicentrifuge M08, ordering number 51-410000
Mini-Incubator IL10, ordering number 51-410100
RiboFlow manipulation plate, ordering number 51-410110

13. Literature

Zhu, S., Schnell, S., and Fischer, M. (2012): Rapid detection of *Cronobacter* spp. with a method combining impedance technology and rRNA based lateral flow assay. International Journal of Food Microbiology 159 (1): 54-58

14. Quick reference protocol

Step		Duration
1.	Pre-enrichment in Buffered Peptone Water	18-24 h
2.	Enrichment or BacTrac analysis	8-24 h
3.	Pre-warm lateral flow assay devices and Solution C at +45°C	
4.	Centrifuge 0.5 ml of enriched sample from BacTrac measuring cell[1] or from enrichment tube[2]	ca. 5 min.
5.	Remove supernatant and resuspend bacteria in 50 µl of Solution A	ca. 1 min.
6.	Add 25 µl of Solution B, mix and incubate at ambient temperature	ca. 5 min.
7.	Add 60 µl of pre-warmed Solution C and mix	ca. 0.5 min.
8.	Apply sample to pre-warmed RiboFlow *Cronobacter* lateral flow assay device and run assay at +45°C	max. 15 min.
9.	Evaluate result immediately	

[1]Only BacTrac-reactive (positive) samples are analysed
[2]All samples from enrichment tubes are analysed individually


SY-LAB