RiboFlow® P/En Detection Kit

Manual, Version 1, June 2016

Product number 51-425113

24 assays

Store at: +2 to +25°C

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

RiboFlow® P/En Detection Kit

Table of contents

1. General information

1.1 Kit components and storage

Solution A, 2 ml
Solution B, 1 ml
Solution C, 2 ml
RiboFlow® P/En lateral flow assay devices, 24 pcs. (4 × 6)
Reaction tubes, 30 pcs.
Manual

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

1.2 Accessory materials and equipment

- Micropipettes
- Sterile pipette tips
- Materials and media for detection of *Pseudomonas* spp. and/or Enterobacteriaceae
- Incubator for enrichment / cultivation of *Pseudomonas* spp. and/or Enterobacteriaceae
- Mini-Centrifuge (relative centrifugal force at least 2000 × g), SY-LAB Geräte GmbH, product number 51-410000
- Mini-Incubator IL10 (SY-LAB Geräte GmbH, product number 51-410100 or VWR international, product number 390-0384)
- RiboFlow® manipulation plate (SY-LAB Geräte GmbH, product number 51-410110)

1.3 Product use / Scope

The RiboFlow® P/En Detection Kit can be used for the detection of *Pseudomonas* spp. and/or Enterobacteriaceae using

- liquid cultures in enrichment media, e.g. according to ISO 21528-1
- sub-cultures of enrichments or of single colonies
- single colonies from (selective) agar plates, e.g. according to ISO 21528-1

This kit is not approved for clinical use. During performance of the test protocol, all due care and attention should be exercised in handling kit components (see chapter 1.4 “Safety information“).

1.4 Safety information

For safe handling of RiboFlow® kit components, please refer to the respective Material Safety Data Sheets. These are available on our website (www.sylab.com) for download by registered users (Microbiology, Service & Downloads / Molecular Microbiology section). 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: *Follow your national safety regulations for handling of microorganisms and take the appropriate measures to prevent infections. Inactivate contaminated material by disinfection and autoclaving.*

1.5 Product warranty and limitation of warranty

SY-LAB Geräte GmbH guarantees the performance of this product as described in chapter 1.7 “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.

1.6 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 (Microbiology, Service & Downloads / Molecular Microbiology section).

1.7 Specifications/Performance

This test kit was developed to enable the detection of *Pseudomonas* spp. and Enterobacteriaceae from enrichment cultures and the confirmation/differentiation of suspicious single colonies from (selective) agar plates either as members of the genus *Pseudomonas* or of the Enterobacteriaceae family, respectively.
A consistent analytical limit of detection of 2.8×10^{10} copies (Enterobacteriaceae) and 3.2×10^{10} copies (*Pseudomonas* spp.) of the respective target nucleic acid molecule is verified for each lot of RiboFlow® P/En Detection Kit.

1.8 Customer service

For technical advice, please contact our customer service (E-mail: supportbio@sylab.com, phone: +43-2231-62252-0, fax: +43-2231-62193).
As our customer you are a valuable source of information concerning your 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.

1.9 Introduction

Some representatives of the so-called “bile-tolerant Gram negative bacteria”, especially pseudomonads, are morphologically not distinguishable from Enterobacteriaceae on VRBG (Violet Red Bile Glucose) agar plates. The confirmation as *Pseudomonas* spp. or as a member of the Enterobacteriaceae, respectively, is then 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. A rapid method based on molecular biology considerably reduces time and costs, in addition often combined with enhanced specificity compared to microbiological/biochemical methods. The RiboFlow® P/En Detection Kit was developed to enable highly specific yet very simple detection of bacteria from the Enterobacteriaceae family and of pseudomonads, and their discrimination, within just a few minutes, with little effort and equipment.

1.10 Test principle

Ribosomal RNA sequences specific for *Pseudomonas* spp. and for the Enterobacteriaceae are detected by a proprietary nucleic acid hybridisation protocol in a simple lateral flow assay format within 30 minutes, using a crude cell extract from an enriched culture or a single colony. Tedious nucleic acid purification or enzymatic amplification of target sequence is not necessary.

2. Protocol

Note: This assay is intended for the analysis of enriched samples (e.g., enrichments according to ISO 21528-1) and/or for the confirmation of suspicious single colonies from (selective) agar plates (e.g., according to ISO 21528-1 or -2).

2.1 Enrichment / cultivation

2.1.1 Enrichment according to ISO 21528-1

Enrichment in buffered peptone water is done according to ISO 21528-1. After enrichment, the RiboFlow® P/En standard protocol for liquid cultures is carried out as described in section 2.2.1 (please observe the important notes in sections 2.2 and 2.4).

2.1.2 Suspicious single colonies

Streaking of samples on VRBG selective plates is done according to ISO 21528-1. Plating of samples by pouring VRBG plates, followed by streaking of suspicious colonies from VRBG on nutrient agar is done according to ISO 21528-2 (**note:** colonies from poured plates cannot be used directly for testing with RiboFlow®, because of the presence of agar in the sample).
Single colonies with a diameter > 2 mm can be taken directly from the agar plate and be tested using the RiboFlow® standard protocol for direct testing of single colonies as described in section 2.2.2, provided the plate was not incubated longer than the specified incubation time, or stored for prolonged time (please observe the important notes in sections 2.2 and 2.4).
If the plate is older and/or a colony to be tested is smaller, sub-cultivation of the suspicious colony is necessary (transfer of the single colony to 0.5 ml of a non-selective medium in a sterile reaction tube, incubation at +35 ± 2°C for 3 ± 1 hours and testing as described in section 2.2.1, beginning with the centrifugation step, or streaking on fresh plate and testing according to section 2.2.2). Such a cultivation step will also allow for isolation of the bacterium. A colony taken from the (selective) plate and tested directly will not be available for isolation any more.

2.2 RiboFlow® P/En lateral flow assay

Note: The measures for compliance with incubation / reaction conditions described in this protocol must be followed, otherwise results can be falsified. This includes the use of an IL-10 Mini-Incubator set to +48°C, the use of a pre-warmed RiboFlow® manipulation plate, as well as pre-warming of RiboFlow® lateral flow devices (test cassettes) and Solution C. Carry out manipulations swiftly and as far as possible inside of the Mini-Incubator with the door open, keep manipulations outside of the incubator (only with manipulation plate) to a minimum. Evaluation of the test result must be carried out immediately after lapse of the specified assay incubation time, before the lateral flow test cassettes can cool to ambient temperature.

2.2.1 Standard protocol for liquid cultures

Note: This protocol is suitable for enrichments according to ISO 21528-1 and for other liquid cultures from non-selective media (e.g., sub-cultures of older / stored enrichments or of single colonies).

Protocol:

1. Set temperature of the IL-10 Mini-Incubator to +48°C and let incubator heat up until +48°C is stably displayed.

2. Pre-warm RiboFlow® manipulation plate in the incubator at +48°C for at least one hour before carrying out the assay.

Note: *The manipulation plate can also remain permanently in the (turned on) IL-10 Mini-Incubator instead of a shelf. In this case, you can start immediately with step 3. If the incubator was turned off, you have to wait one hour after +48°C is displayed stably before the assay is carried out, to ensure that the plate will also reach the required temperature.*

3. Pre-warm Solution C and the required number of RiboFlow® lateral flow assay devices (test cassettes) for at least 10 minutes in the IL-10 Mini-Incubator set to +48°C, before the assay is carried out. To this end, slide the devices into the pre-warmed RiboFlow® manipulation plate lying in the incubator, so that the sample ports are not covered by the acrylic glass cover of the plate (Figure 1).
Bring the other kit components / solutions to ambient temperature (+18 to +30°C) before use.

4. After cultivation, agitate liquid cultures gently (do not spill) or homogenise by pipetting up and down before taking samples for this test. For 0.5 ml sub-cultures of single colonies, start with the centrifugation step (step 6).

5. Transfer 0.5 ml of homogenised sample to a reaction tube.

6. Centrifuge bacteria for 5 minutes at a minimum of 2000 × g.

7. Carefully remove and discard supernatant without losing the bacterial pellet.

8. Resuspend bacterial pellet thoroughly but carefully in 50 µl of Solution A by pipetting up and down, avoid foaming.

9. Add 25 µl of Solution B to the sample and mix well (vortex if possible). Now the bacterial pellet must be completely re-suspended.

Note: *If a vortex is available and several bacterial pellets have to be processed simultaneously, it may be more convenient and time-saving to add 75 µl each of a prepared 2 + 1 mixture of Solution A + Solution B to all samples first, and then to vortex the bacterial pellets thoroughly for resuspension.*

10. Incubate homogenate at ambient temperature (+18 to +30°C) for 6 ± 1 minutes.

11. Add 60 µl of the pre-warmed Solution C to the sample and mix (vortex if possible). Proceed immediately with step 12.

12. Quickly apply the entire sample (~135 µl) to the sample port of a pre-warmed RiboFlow® lateral flow assay device on the manipulation plate. Let the sample penetrate the sample application pad. Handle device / manipulation plate carefully after application of sample to avoid spillage.

Notes: *Perform all necessary manipulations with the IL-10 Mini-Incubator nearby and avoid removing the RiboFlow® manipulation plate from the incubator before/during the assay. To avoid cooling of the plate and/or test cassettes before running the assay, the application of samples can be done conveniently and swiftly after sliding the plate slightly outwards on its mountings, with the incubator door open.*
Migration of the sample along the RiboFlow® device can conveniently be monitored through the glass window of the closed incubator door. If the flow does not start within 2 minutes (this can happen from time to time with

extremely viscous samples), it may be helpful to gently scratch the surface of the pad in the sample port with a micropipette tip.

13. Incubate assay(s) on the manipulation plate for 15 ± 1 minutes in the closed IL-10 Mini-Incubator, then evaluate the result immediately (see section 2.3).

2.2.2 Standard protocol for direct testing of single colonies

Notes: This protocol is suitable for direct testing of single colonies (> 2 mm diameter) from plates, without additional sub-culturing in liquid medium.
Please note that a sub-cultivation step as described in section 2.1.2 must be carried out first when plates were incubated too long or stored. Please observe the important notes in sections 2.2 and 2.4 when carrying out the test protocol.

Protocol:

1. Set temperature of the IL-10 Mini-Incubator to +48°C and let incubator heat up until +48°C is stably displayed.

2. Pre-warm RiboFlow® manipulation plate in the incubator at +48°C for at least one hour before carrying out the assay.

Note: *The manipulation plate can also remain permanently in the (turned on) IL-10 Mini-Incubator instead of a shelf. In this case, you can start immediately with step 3. If the incubator was turned off, you have to wait one hour after +48°C is displayed stably before the assay is carried out, to ensure that the plate will also reach the required temperature.*

3. Pre-warm Solution C and the required number of RiboFlow® lateral flow assay devices (test cassettes) for at least 10 minutes in the IL-10 Mini-Incubator set to +48°C, before the assay is carried out. To this end, slide the devices into the pre-warmed RiboFlow® manipulation plate lying in the incubator, so that the sample ports are not covered by the acrylic glass cover of the plate (Figure 1).
Bring the other kit components / solutions to ambient temperature (+18 to +30°C) before use.

4. Prepare a mixture of 50 µl Solution A and 25 µl Solution B in an empty reaction tube.

5. Remove a typical colony from the (selective) agar plate using an inoculation loop and resuspend it thoroughly in the mixture of Solution A and Solution B prepared in paragraph 4.

6. Mix well (vortex if possible) and incubate mixture at ambient temperature (+18 to +30°C) for 6 ± 1 minutes.

7. Add 60 µl of the pre-warmed Solution C to the sample and mix. Proceed immediately with step 8.

8. Quickly apply the entire sample (~135 µl) to the sample port of a pre-warmed RiboFlow® lateral flow assay device on the manipulation plate. Let the sample penetrate the sample application pad. Handle device / manipulation plate carefully after application of sample to avoid spillage.

Notes: *Perform all necessary manipulations with the IL-10 Mini-Incubator nearby and avoid removing the RiboFlow® manipulation plate from the incubator before/during the assay. To avoid cooling of the plate and/or test cassettes before running the assay, the application of samples can be done conveniently and swiftly after sliding the plate slightly outwards on its mountings, with the incubator door open.*
Migration of the sample along the RiboFlow® device can conveniently be monitored through the glass window of the closed incubator door. If the flow does not start within 2 minutes (this can happen from time to time with extremely viscous samples), it may be helpful to gently scratch the surface of the pad in the sample port with a micropipette tip.

9. Incubate assay(s) on the manipulation plate for 15 ± 1 minutes in the closed IL-10 Mini-Incubator, then evaluate the result immediately (see section 2.3).

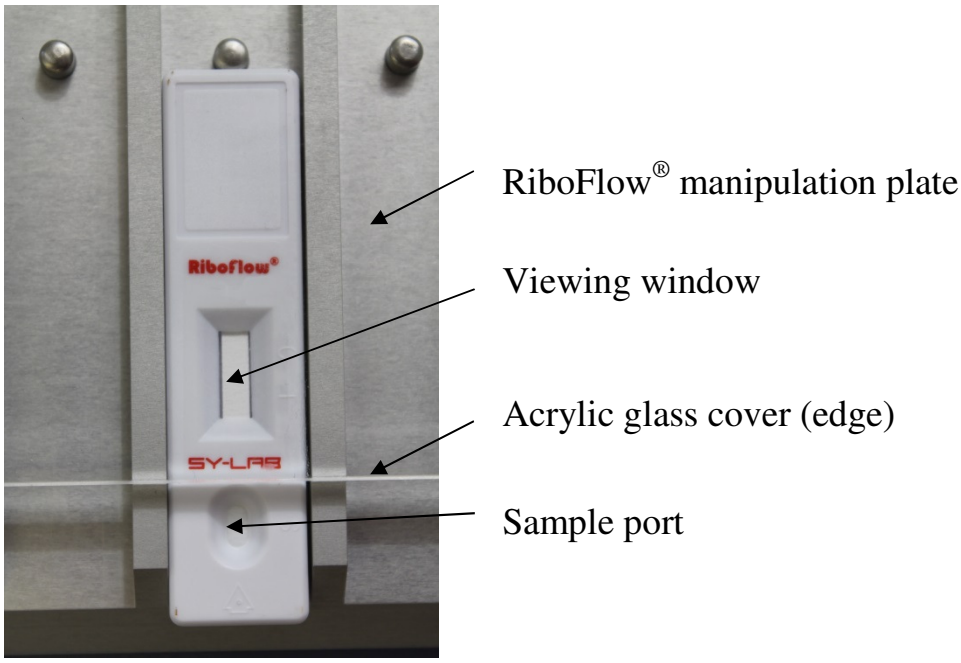


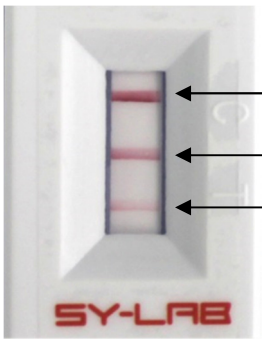
Fig. 1: RiboFlow® lateral flow assay device

2.3 Evaluation

Note: RiboFlow® lateral flow assays must be evaluated **immediately** after 15 ± 1 minutes runtime. Runtimes >16 minutes might lead to false positive results, especially when the temperature during the run was too low. Unspecific lines may also appear a few minutes after the test is finished and is cooling to ambient temperature.

Evaluation: Figs. 2 - 4 show viewing windows displaying possible results of RiboFlow® P/En lateral flow assays.

a)

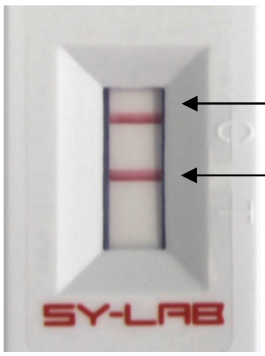


Control line (top)

Test line (Enterobacteriaceae-specific, middle)

Test line (*Pseudomonas sp.*-specific, bottom)

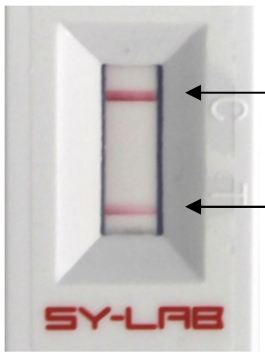
b)



Control line (top)

Test line (Enterobacteriaceae-specific, middle)

c)



Control line (top)

Test line (*Pseudomonas sp.*-specific, bottom)

Fig. 2: Positive Results:
a) If the control line (top), as well as the two test lines (the Enterobacteriaceae – specific test line in the middle and the *Pseudomonas sp.* – specific test line at the bottom), are visible in the viewing window, the result is positive for both organism groups.
b) If the control line (top) appears together with the Enterobacteriaceae – specific test line in the middle, the result is positive for Enterobacteriaceae.
c) If the control line (top) appears together with the *Pseudomonas sp.* – specific test line at the bottom, the result is positive for *Pseudomonas sp.*

In strongly positive samples, the control line (top) can be very faint.

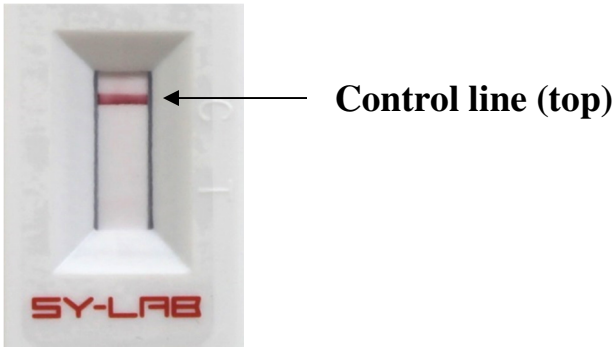


Fig. 3: Negative result:
If only the control line (top) is visible, no Enterobacteriaceae or *Pseudomonas sp.* could be detected with the assay, and the result is negative.

Fig. 4: Invalid result: If no lines are visible at all, some error has occurred during processing of the assay. Such a result is invalid, and the test has to be repeated starting from section 2.2, using a new RiboFlow® lateral flow assay device.

To file a permanent record of results (if desired), we recommend photography using a digital camera immediately at the end of the runtime. Since the acrylic glass cover of the RiboFlow® manipulation plate is transparent, the devices can be conveniently left in the plate for photography. This will also delay cooling of the devices and thus the potential danger of formation of false positive signals after the run.

2.4 Important general notes

- This assay should always be carried out with freshly grown bacteria at the end of the specified incubation time for the respective culture. It should not be used with stored samples or samples that were incubated for too long, since rRNA may be degraded during prolonged incubation or storage, potentially declining to undetectable levels. However, the rest of an enriched sample can be stored refrigerated for a few hours, until a result is available, to enable a second analysis on the same day, if necessary.
  If longer storage of an enriched sample or a plate is unavoidable, a sub-culture in non-selective medium should be carried out to boost rRNA synthesis prior to analysis.
  Bacterial pellets after centrifugation or bacteria re-suspended in Solution A may be stored as pellets or lysates at -20°C for prolonged time (a few weeks).

- Always agitate enriched cultures gently without spilling, or homogenise by pipetting up and down before taking a sample.

- The bacterial pellet must be completely and homogenously re-suspended after mixing with Solution B!

- 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 centrifuge.

- When several samples are analysed simultaneously, 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.

- 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. This RiboFlow® test kit was developed and evaluated under the conditions stated in this manual, using an IL-10 Mini-Incubator and a RiboFlow® manipulation plate. If other incubators, which have not been specifically qualified, are used, false positive results may occur.

- Lateral flow assays must be evaluated **immediately** and **quickly** after lapse of the specified runtime, since cooling to ambient temperature may falsify negative results.

- Always work with sterile pipette tips to avoid microbial or nuclease contamination of kit components.

- RiboFlow® **video tutorials** are available on our website (www.sylab.com, Microbiology, Service & Downloads / Molecular Microbiology section) for proper guidance! If you have further questions concerning this kit, the SY-LAB customer support will be glad to assist you.

3. Ordering information

- **RiboFlow® P/En Detection Kit**, 24 assays, product number 51-425113
- **Mini-Centrifuge M08**, product number 51-410000
- **Mini-Incubator IL10**, product number 51-410100
- **RiboFlow® manipulation plate**, product number 51-410110

4. Quick reference protocols

4.1 Quick reference protocol for liquid cultures

Step		Duration
1.	Enrichment	depending on method
2.	Pre-warm lateral flow assay devices (on the pre-warmed RiboFlow® manipulation plate) and Solution C for at least 10 min in an IL-10 Mini-Incubator set to +48°C. Bring other kit components to ambient temperature (+18 to +30°C).	
3.	Centrifuge 0.5 ml of enriched sample	~5 min
4.	Remove supernatant and resuspend bacteria in 50 µl of Solution A	~1 min
5.	Add 25 µl of Solution B, mix and incubate at ambient temperature (+18 to +30°C)	6 ± 1 min
6.	Add 60 µl of pre-warmed Solution C and mix	~0.5 min
7.	Apply entire sample (~135 µl) to pre-warmed RiboFlow® lateral flow assay device on the manipulation plate in the IL-10 Mini-Incubator set to +48°C and incubate	15 ± 1 min
8.	Evaluate result immediately	

4.2 Quick reference protocol for direct testing of single colonies

Step		Duration
1.	Streak and incubate plate	depending on method
2.	Pre-warm lateral flow assay devices (on the pre-warmed RiboFlow® manipulation plate) and Solution C for at least 10 min in an IL-10 Mini-Incubator set to +48°C. Bring other kit components to ambient temperature (+18 to +30°C).	
3.	Prepare a mixture of 50 µl Solution A and 25 µl of Solution B in a reaction tube	~1 min
4.	Thoroughly resuspend a single colony (> 2 mm diameter) in the prepared mixture	~0.5 min
5.	Incubate at ambient temperature (+18 to +30°C) for 6 ± 1 minutes	6 ± 1 min
6.	Add 60 µl of pre-warmed Solution C and mix	~0.5 min
7.	Apply entire sample (~135 µl) to pre-warmed RiboFlow® lateral flow assay device on the manipulation plate in the IL-10 Mini-Incubator set to +48°C and incubate	15 ± 1 min
8.	Evaluate result immediately	


SY-LAB