



# **RiboFlow<sup>®</sup> *Listeria Twin* Detection Kit**

Manual, Version 2, December 2014

Product number 51-419113

**24 Assays**

Store at +2 to +25°C

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# RiboFlow<sup>®</sup> *Listeria Twin* Detection Kit

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# 1. General Information

## 1.1 Kit components and storage

Solution A, 2 ml

Solution B, 1 ml

Solution C, 2 ml

RiboFlow<sup>®</sup> *Listeria Twin* Lateral Flow Assay device, 24 pcs. (4 × 6)

Reaction tubes, 30 pcs.

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

## 1.2 Accessory materials and equipment

Required:

- Micropipettes
- Sterile pipette tips
- Sterile stomacher bags with lateral filter, 400 ml, bag clips and lab blender (e. g. Stomacher<sup>®</sup>)
- Incubator for pre-enrichment / enrichment
- Microcentrifuge (relative centrifugal force at least 2000 × g), SY-LAB Geräte GmbH, product number 51-410000
- Incubator for RiboFlow<sup>®</sup> procedure: Highly recommended for reliable temperature compliance during RiboFlow<sup>®</sup> protocol: Small incubator with viewing window (Mini-Incubator IL10, SY-LAB Geräte GmbH, product number 51-410100)
- Sterile (test) tubes, volume 10 ml or more
- BiMedia 404A (ONE Broth-Listeria) dehydrated culture medium + selective supplement for pre-enrichment and enrichment, refer to section 3

Optional / recommended:

- BacTrac impedance analyser and disposable sterile measuring cells with 4 electrodes (contact SY-LAB Geräte GmbH for further information)
- Non-selective medium such as Brain Heart Infusion Broth (BHI), and additional sterile 1.5 ml reaction tubes (for sub-cultivations of older cultures and/or cultivation + confirmation of suspicious single colonies from selective agar plates, if appropriate)

- RiboFlow<sup>®</sup> manipulation plate, SY-LAB Geräte GmbH, product number 51-410110

### 1.3 Product use / Scope

The RiboFlow<sup>®</sup> *Listeria Twin* Detection Kit can be used to detect *Listeria monocytogenes* / *Listeria sp.* in food and environmental samples using

- selective enrichment cultures in BiMedia 404A (ONE Broth-Listeria), with or without impedance analysis
- liquid cultures in non-selective media (sub-cultures of enrichments or of single colonies)
- single colonies (diameter > 2 mm) from agar plates

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

When handling with RiboFlow<sup>®</sup> kit components, please refer to the respective Material Safety Data Sheets. These are available on our website ([www.sylab.com](http://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:*** *Listeria monocytogenes* is a human pathogen. 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.

### 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](http://www.sylab.com)) for download by registered users (Microbiology, Service & Downloads / Molecular Microbiology section).

## 1.7 Specifications/Performance

This test kit was developed to ensure detection of  $\geq 1$  cfu / 25 g product after enrichment according to the protocols described.

An analytical limit of detection of  $3 \times 10^{10}$  copies of the target nucleic acid molecules is verified for each lot of RiboFlow<sup>®</sup> *Listeria Twin* Detection Kit.

## 1.8 Customer service

For technical advice, please contact our customer service (E-mail: [supportbio@sylab.com](mailto: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

Routinely, the detection of *Listeria monocytogenes*/*Listeria sp.* 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. A rapid method for confirmation of positive screening results based on molecular biology considerably reduces time and costs, in addition often combined with enhanced specificity compared to

microbiological/biochemical methods. The RiboFlow<sup>®</sup> *Listeria Twin* Detection Kit was developed to enable highly specific but at the same time economically priced detection of *Listeria monocytogenes* and other *Listeria* species within just a few minutes, with little hands-on time and without additional costly equipment.

### **1.10 Test principle**

With RiboFlow<sup>®</sup> *Listeria Twin*, a *Listeria monocytogenes*-specific ribosomal RNA sequence and another rRNA sequence specific for all *Listeria* species are detected simultaneously by a proprietary nucleic acid hybridisation protocol in a simple lateral flow assay format, 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 (selective enrichment or impedance analysis). It can also be used for the confirmation of suspicious single colonies from (selective) agar plates.

### 2.1 Media preparation and pre-enrichment

For information regarding preparation of BiMedia 404A (=ONE Broth-Listeria + selective supplement) and *Listeria* screening with the BacTrac impedance method, please follow the specific SY-LAB media preparation instructions and BacTrac application notes for impedance detection of *Listeria* on our website ([www.sylab.com](http://www.sylab.com), Microbiology, Service & Downloads / Electrical Microbiology and Molecular Microbiology sections).

1. Prepare the required volume of BiMedia 404A (= ONE Broth-Listeria + selective supplement) for pre-enrichment and enrichment according to the instructions on the package or the media preparation instruction for BiMedia 404A. For each sample, 225 ml for pre-enrichment and 1 × 9 ml for enrichment / BacTrac analysis are required.

**Note:** Prepared BiMedia 404A can be stored at +2 to +8°C for up to 48 hours.

2. Per sample, fill either one sterile test tube (for enrichment without impedance analysis), or one sterile disposable measuring cell with 4 electrodes (for BacTrac analysis) with 9 ml of BiMedia 404A. The remaining medium will be needed for pre-enrichments. The tubes / measuring cells are stored at +2 to +8°C until use on the next day.
3. Pre-warm the medium to  $+30 \pm 1^\circ\text{C}$  before use.
4. Weigh 25 g of sample into a sterile lab blender bag with filter and add 225 ml of pre-warmed BiMedia 404A.
5. Close bag and homogenise sample for 30 seconds using a stomacher.
6. Incubate samples at  $+30 \pm 1^\circ\text{C}$  for 22-24 h.



## 2.2 Enrichment / BacTrac Screening

### 2.2.1 Enrichment without impedance analysis

1. Pre-warm prepared tubes with BiMedia 404A to  $+30 \pm 1^\circ\text{C}$ .
2. Homogenise pre-enriched sample in bag by kneading and transfer 1 ml of sample from the filtered part of the bag to a pre-warmed tube.
3. Incubate sample at  $+30 \pm 1^\circ\text{C}$  for 22-24 h.
4. Subsequently, carry out standard protocol for RiboFlow<sup>®</sup> *Listeria Twin* as described in section 2.3.1 (please observe the important notes in sections 2.3 and 2.5).

### 2.2.2 BacTrac screening (impedance analysis)

1. Homogenise pre-enriched sample in bag by kneading and transfer 1 ml of sample from the filtered part of the bag to a prepared BacTrac measuring cell with BiMedia 404A.
2. Carry out BacTrac analysis according to respective SY-LAB application note.
3. When the BacTrac analysis is finished, carry out standard protocol for RiboFlow<sup>®</sup> *Listeria Twin* as described in section 2.3.1 (please observe the important notes in sections 2.3 and 2.5). Only BacTrac-reactive, i.e. positive, samples are analysed.

***Notes:*** *This test 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.*

### 2.2.3 Single colonies from agar plates

1. Streak bacteria on (selective) agar plates and incubate plates according to the method used.
2. Either analyse a big colony (> 2 mm) directly from the plate right after the 24 h incubation step as described in protocol 2.3.2 (please observe the important notes in sections 2.3 and 2.5), or perform a short sub-cultivation in a non-selective medium first, as described in paragraph 3 below, if the colonies are too small and/or the plate is older.
3. With a sterile inoculation loop, 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. BHI medium), and incubate for 2-3 hours at  $+37 \pm 1^\circ\text{C}$ . Streak a loopful on a new plate for isolation, if desired.
4. Carry out RiboFlow<sup>®</sup> *Listeria Twin* standard protocol according to 2.3.1, starting with the centrifugation step in paragraph 4 (please observe the important notes in sections 2.3 and 2.5).

***Notes:*** Colonies with a diameter > 2 mm can be tested directly if desired, provided the plate is not older than 24 hours.

*If the plate is older than 24 hours and/or the colony is smaller, we recommend a short sub-cultivation of single colonies in liquid medium. Such a cultivation step will also allow for isolation of the bacterium by streaking it from the liquid culture to a new plate before the rest of the culture is centrifuged for analysis. A colony taken from the plate and tested directly will not be available for isolation any more.*

## 2.3 RiboFlow<sup>®</sup> *Listeria* Twin Lateral Flow Assay

### Important notes:

- *For the SY-LAB IL-10 Mini-Incubator, we recommend to set the incubator temperature to +48°C to achieve the recommended assay temperature of  $+46 \pm 1^\circ\text{C}$  inside the lateral flow devices.*
- *When using a RiboFlow<sup>®</sup> manipulation plate, pre-warm the plate in the incubator for at least 1 h before the assay is carried out, to ensure correct reaction temperature during test performance!*
- *Solution C and RiboFlow<sup>®</sup> Lateral Flow Assay devices must be pre-warmed to  $+46 \pm 1^\circ\text{C}$  for at least 10 minutes before the assay is carried out.*
- *Solution A and Solution B must be brought to ambient temperature (+18 to +30°C) before use.*
- *After addition of Solution B, the recommended incubation/reaction conditions must be followed, otherwise erroneous results may occur.*

### 2.3.1 Standard protocol for liquid cultures

This protocol is suitable for selective enrichments and for liquid cultures from non-selective media, e.g. subcultures of older / stored enrichments or of single colonies.

1. Pre-warm Solution C and the required number of RiboFlow<sup>®</sup> Lateral Flow Assay devices to  $+46 \pm 1^\circ\text{C}$  for at least 10 minutes before the assay is carried out. The other kit components must be used at ambient temperature (+18 to +30°C). A tray or similar (e.g. RiboFlow<sup>®</sup> manipulation plate) is convenient to handle several tests at once.
2. After cultivation, agitate liquid cultures gently (do not spill) or homogenise by pipetting up and down before taking samples for this test. For subcultures of single colonies, start with the centrifugation step (paragraph 4 below).

3. Transfer 0.5 ml of homogenised sample to a reaction tube.
4. Centrifuge bacteria for 5 minutes at a minimum of  $2000 \times g$ .
5. Carefully remove and discard supernatant without losing the bacterial pellet.
6. Resuspend bacterial pellet thoroughly but carefully in 50  $\mu$ l of Solution A by pipetting up and down, avoid foaming.
7. Add 25  $\mu$ l of Solution B to the sample and mix thoroughly (vortex if possible). Now the bacterial pellet must be completely resuspended.
8. Incubate mixture at ambient temperature (+18 to +30°C) for 5 minutes.
9. Add 60  $\mu$ l of the pre-warmed (+46  $\pm$  1°C) Solution C to the sample and mix by pipetting up and down. Proceed immediately with step 10.

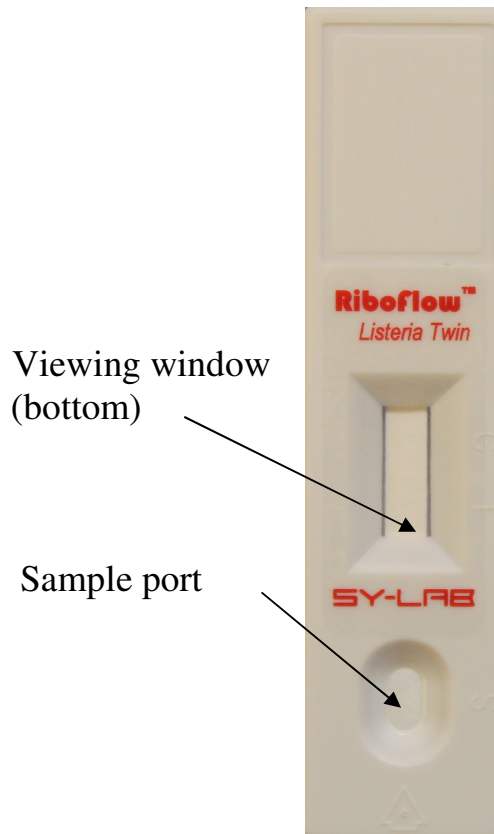
**Note:** *If a vortex is available and several bacterial pellets have to be processed simultaneously, it may be more convenient and time-saving to add Solution A and Solution B successively to all samples first, and then to vortex the bacterial pellets thoroughly for resuspension prior to incubation at ambient temperature (+18 to +30°C).*

10. Quickly apply the entire sample (~135  $\mu$ l) to the sample port of a pre-warmed (+46  $\pm$  1°C) RiboFlow<sup>®</sup> *Listeria Twin* Lateral Flow Assay device (Fig. 1) resting horizontally on a flat surface. Let the sample penetrate the sample application pad. Handle device carefully after application of sample to avoid spillage.
11. Incubate the assay for a maximum of 15 minutes at +46  $\pm$  1°C, then evaluate the result immediately (see section 2.4).

### 2.3.2 Standard protocol for direct testing of single colonies

This protocol is suitable for direct testing of single colonies (> 2 mm diameter) from plates without additional liquid subculture.

1. Pre-warm Solution C and the required number of RiboFlow<sup>®</sup> Lateral Flow Assay devices to  $+46 \pm 1^\circ\text{C}$  for at least 10 minutes before the assay is carried out. The other kit components must be used at ambient temperature (+18 to  $+30^\circ\text{C}$ ). A tray or similar (e.g. RiboFlow<sup>®</sup> manipulation plate) is convenient to handle several tests at once.
2. Prepare a mixture of 50  $\mu\text{l}$  Solution A and 25  $\mu\text{l}$  Solution B in an empty reaction tube.
3. Remove a typical colony from a (selective) agar plate using an inoculation loop and resuspend it thoroughly in the mixture of 50  $\mu\text{l}$  of Solution A and 25  $\mu\text{l}$  solution B prepared in paragraph 2.
4. Vortex if possible and incubate mixture at ambient temperature (+18 to  $+30^\circ\text{C}$ ) for 5 minutes.
5. Add 60  $\mu\text{l}$  of the pre-warmed ( $+46 \pm 1^\circ\text{C}$ ) Solution C to the sample and mix by pipetting up and down. Proceed immediately with step 6.
6. Quickly apply the entire sample (~135  $\mu\text{l}$ ) to the sample port of a pre-warmed ( $+46 \pm 1^\circ\text{C}$ ) RiboFlow<sup>®</sup> *Listeria Twin* Lateral Flow Assay device (Fig. 1) resting horizontally on a flat surface. Let the sample penetrate the sample application pad. Handle device carefully after application of sample to avoid spillage.
7. Incubate the assay for a maximum of 15 minutes at  $+46 \pm 1^\circ\text{C}$ , then evaluate the result immediately (see section 2.4).

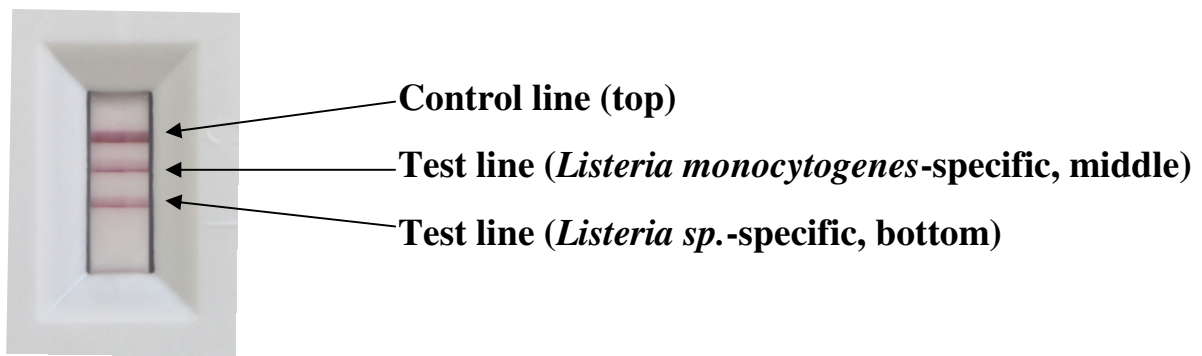


**Fig. 1: RiboFlow® *Listeria Twin* Lateral Flow Assay device**

## 2.4 Evaluation

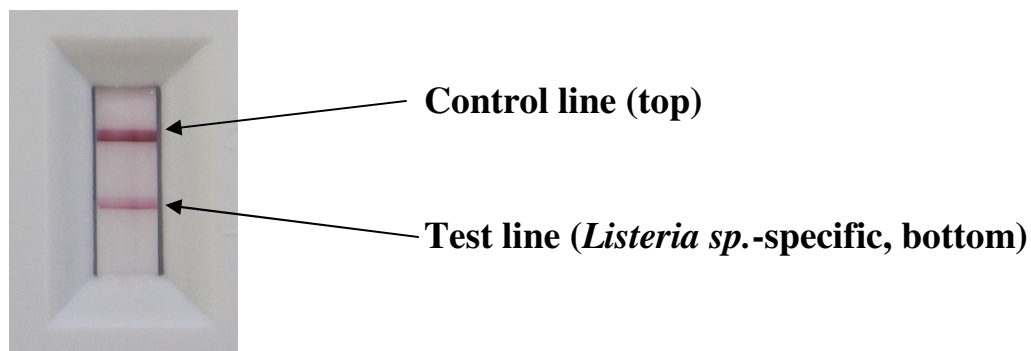
**Note:** *RiboFlow® Listeria Twin must be evaluated immediately after 15 minutes runtime at  $+46 \pm 1^\circ\text{C}$ . Runtimes  $>15$  minutes might lead to false positive results, especially at temperatures  $<+45^\circ\text{C}$ . Unspecific bands may appear also a few minutes after the test is finished and is cooling to ambient temperature.*

**Evaluation:** Figs. 2 - 5 are showing viewing windows displaying possible results of RiboFlow® *Listeria Twin*.



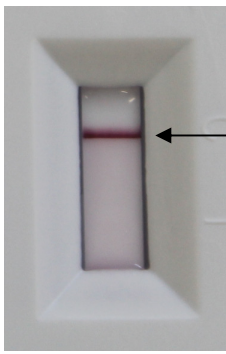
**Fig. 2: Positive result for *Listeria monocytogenes*:** Only if all three lines, i.e. control line (top), *Listeria monocytogenes*-specific test line (middle) and *Listeria sp.*-specific test line (bottom) are visible in the viewing window, the result is positive for *Listeria monocytogenes*. Sometimes the control line (top) is very faint in strongly positive samples.

**Note:** *Listeria marthii*, a species very closely related to *L. monocytogenes* and *L. innocua*, cannot be distinguished from *L. monocytogenes* by RiboFlow® *Listeria Twin* due to identical rRNA sequences



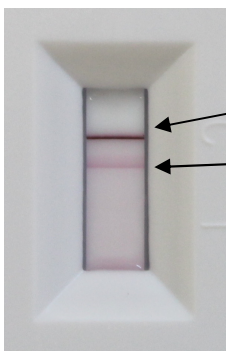
**Fig. 3: Positive result for *Listeria sp.* other than *L. monocytogenes*:** If the control line (top) and the *Listeria sp.*-specific test line (bottom) are visible – **without** the *L. monocytogenes* – specific test line (middle), the result is positive for *Listeria sp.*, i.e., some *Listeria* species other than *L. monocytogenes*/*L. marthii* is present.

a)



Control line (top)

b)



Control line (top)

Test line (*Listeria monocytogenes*-specific, middle)

**Fig. 4: Negative results:**

a) If **only** the control line (top) is visible, *Listeria monocytogenes* or other *Listeria sp.* could not be detected with the assay, and the result is negative.

b) The result is also negative when the control line (top) is appearing together with the *L. monocytogenes* – specific test line (middle), **but without** the *Listeria sp.* – specific test line (bottom).



**Fig. 5: 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 starting from section 2.3, using a new RiboFlow<sup>®</sup> 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.

## 2.5 Important general notes

- This assay should always be carried out with freshly grown cultures at the end of the specified incubation time for the respective enrichment/culture. It should not be used with stored/old samples or enrichments/plates that were incubated for too long, since rRNA may be degraded quickly during prolonged incubation or storage, potentially declining to undetectable levels. However, the rest of an enriched sample (BacTrac 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. If longer storage of an enriched sample is unavoidable, a subculture using a non-selective medium should be performed for 4-5 hours to boost rRNA synthesis prior to analysis.  
Bacterial pellets after centrifugation or bacteria resuspended in Solution A may be stored as pellets or lysates at  $-20^{\circ}\text{C}$  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 homogeneously resuspended after mixing with Solution B!
- Centrifugations must be carried out for at least 5 minutes with a relative centrifugal force (RCF) of at least  $2000 \times g$  (up to  $5000 \times g$ ) to ensure sedimentation of bacteria. Information regarding the RCF can be found in the user manual of your microcentrifuge.
- 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. Therefore, it is **absolutely essential to run the RiboFlow<sup>®</sup>**

**Listeria Twin lateral flow assay at  $+46 \pm 1^\circ\text{C}$ .** The use of a mini-incubator with viewing window (with the temperature of the incubator set to  $+48^\circ\text{C}$ ) in combination with a pre-warmed (!) RiboFlow<sup>®</sup> manipulation plate (both available at SY-LAB Geräte GmbH), to ensure a reaction temperature in the test device of  $+46 \pm 1^\circ\text{C}$ , is highly recommended. Lateral flow tests must be evaluated **immediately** and **quickly** after 15 minutes runtime, because false positive signals may arise after completion of the test during cooling to ambient temperature.

- Always work with sterile pipette tips to avoid microbial or nuclease contamination of kit components.
- RiboFlow<sup>®</sup> **video tutorials** are available on our website ([www.sylab.com](http://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<sup>®</sup> Listeria Twin Detection Kit**, 24 assays, product number 51-419113
- **BiMedia 404A (ONE Broth-Listeria) dehydrated culture medium**, 500 g + 22 vials of ONE Broth-Listeria Selective Supplement, for ~12 l, product number 41-472200
- **Disposable empty measuring cells with 4 electrodes**, gamma-irradiated, 400 pcs., product number 41-440004
- **Mini-Centrifuge M08**, product number 51-410000
- **Mini-Incubator IL10**, product number 51-410100
- **RiboFlow<sup>®</sup> manipulation plate**, product number 51-410110

## 4. Quick reference protocols

### 4.1 Quick reference protocol for liquid cultures

<u>Step</u>	<u>Duration</u>
1. Pre-enrichment	22-24 hrs
2. Enrichment or BacTrac analysis	22-24 hrs
3. Pre-warm lateral flow assay devices and Solution C to $+46 \pm 1^\circ\text{C}$	
4. Centrifuge 0.5 ml of enriched sample from measuring cell <sup>1</sup> or from enrichment tube <sup>2</sup>	~5 min
5. Remove supernatant and resuspend bacteria in 50 $\mu\text{l}$ of Solution A	~1 min
6. Add 25 $\mu\text{l}$ of Solution B, mix and incubate at ambient temperature ( $+18$ to $+30^\circ\text{C}$ )	~5 min
7. Add 60 $\mu\text{l}$ of <u>pre-warmed Solution C</u> and mix	~0.5 min
8. Apply entire sample (apprx. 135 $\mu\text{l}$ ) to <u>pre-warmed RiboFlow<sup>®</sup> <i>Listeria Twin</i> Lateral Flow Assay device</u> and <u>incubate at <math>+46 \pm 1^\circ\text{C}</math></u>	max. 15 min
9. Evaluate result immediately	

<sup>1</sup>**Only BacTrac-reactive (positive) samples are analysed**

<sup>2</sup>**All samples are analysed**

## 4.2 Quick reference protocol for direct testing of single colonies

<u>Step</u>	<u>Duration</u>
1. Streak and incubate plate acc. to method used	24 hrs
2. Pre-warm lateral flow assay devices and Solution C to $+46 \pm 1^\circ\text{C}$ .	
3. Prepare a mixture of 50 $\mu\text{l}$ Solution A and 25 $\mu\text{l}$ of Solution B in a reaction tube	~1 min
4. Thoroughly resuspend a colony ( $> 2$ mm diameter) in the prepared mixture	~0.5 min
5. Incubate at ambient temperature ( $+18$ to $+30^\circ\text{C}$ ) for 5 minutes	~5 min
6. Add 60 $\mu\text{l}$ of <u>pre-warmed Solution C</u> and mix	~0.5 min
7. Apply entire sample (apprx. 135 $\mu\text{l}$ ) to <u>pre-warmed RiboFlow<sup>®</sup> <i>Listeria Twin</i> Lateral Flow Assay device</u> and <u>incubate at <math>+46 \pm 1^\circ\text{C}</math></u>	max. 15 min
8. Evaluate result immediately	

**SY-LAB**