

# LISTERIA SYSTEM 18R

System for biochemical identification of *Listeria spp.*

## DESCRIPTION

**LISTERIA SYSTEM 18R** is a system with 18 wells containing dried biochemical substrates for species identification of *Listeria* genus isolated from cultures in selective or non selective agar media. The system is inoculated with a suspension of *Listeria spp.* to be identified and incubated at 36°C±1°C for 18-24 hours. Microorganism is identified through numerical codification obtained by chromatic toning of the several biochemical test.

## USE

**LISTERIA SYSTEM 18 R** allows identification in 18-24 hours of *Listeria spp.* strains isolated in selective and non selective agar culture media from food samples:

- *Listeria monocytogenes*
- *Listeria innocua*
- *Listeria grayi subsp. grayi*
- *Listeria grayi subsp. murrayi*
- *Listeria seeligeri*
- *Listeria welshimeri*
- *Listeria ivanovii*
- *Jonesia denitrificans*

## KIT CONTENTS

The kit contains:

- 20 Systems **LISTERIA SYSTEM 18 R**
- 20 Vials Physiological Solution
- 20 Xylose Disc
- 20 ABN Disc
- 1 Vial ABN Reagent (3 ml)
- 1 Instruction sheet

## ITEMS NECESSARY BUT NOT INCLUDED IN THE KIT

- **LISTERIA SYSTEM 18 R – Reagent** (code 80257)
- **GRAM COLOR KIT** (code 80293)
- Sundry microbiology laboratory materials
- **VASELIN OIL** (code 80278)

## CONFIGURATION

The configuration of the system is shown in Table n°1.

Table n°1

Well	BIOCHEMICAL IDENTIFICATION
<b>1-ONPG</b>	ONPG hydrolysis
<b>2-UR</b>	Urea hydrolysis
<b>3- H<sub>2</sub>S</b>	Production of Sulfurate hydrogen
<b>4-IND</b>	Indole production
<b>5-RAF</b>	Raffinose fermentation
<b>6-CAT</b>	Catalase production
<b>7-MR</b>	Production of organic acid
<b>8-ESC</b>	Aesculine hydrolysis
<b>9-ABN</b>	Aminoacil-β-naftilamide hydrolysis
<b>10-GLU</b>	Glucose fermentation
<b>11-ARL</b>	Arabitole fermentation
<b>12-MAL</b>	Maltose fermentation
<b>13-RAM</b>	Rhamnose fermentation
<b>14-AMDM</b>	α-methyl-D-mannoside fermentation
<b>15-XYL</b>	Xylose fermentation
<b>16-MAN</b>	Mannitol fermentation
<b>17-NIT</b>	Nitrate and nitrite reduction
<b>18-VP</b>	Acetoine production

## MODALITY OF USE

### 1) PREPARATION OF BACTERIAL SUSPENSION

Make sure that the strain under test belongs to *Listeria* genus observing colonies morphology and carrying out a Gram staining.

- Open one vial of saline solution for microbiological use contained in the kit.
- Pick up 2-3 colonies well isolated and equal morphologically from selective or non selective agar culture media for *Listeria spp.* isolation.
- Carefully suspend the bacterial colony in physiological solution.

### 2) INOCULUM OF THE SYSTEM

- Pick up a system from its envelope and take it to room temperature.
- Introduce a disc of xilose, contained in the kit, in well **15-XYL**.
- Note identification details of the sample under test.
- Transfer 0.2 ml ( 4 drops) of bacterial suspension into each well of the system.
- Add to wells **2-UR** and **3-H<sub>2</sub>S**, 2 drops of vaseline oil for microbiological use.
- Cover the system with the appropriate lid and incubate at 36°C ± 1°C for 18-24 hours.

### 3) AFTER INCUBATION, CARRY OUT ADDITIONAL TESTS:

#### • ABN TEST

Introduce a disc of ABN, contained in the kit, in well **9-ABN** and incubate the system at 36°C ± 1°C for further 4 hours. Add 2 drops of ABN Reagent, contained in the kit, and observe the presence or the absence of a yellow coloration.

Appearance of a yellow colouration indicates the presence of *Listeria spp.* not *monocytogenes* (Positive test).

The absence of a yellow colouration may indicate presence of *Listeria monocytogenes* (Negative test).

Confirm the presence of *Listeria monocytogenes* with CAMP TEST S.

#### AFTERWARDS CARRY OUT:

#### • INDOLE TEST

Add 3 drops of Kovac's reagent in well **4-IND**, wait for 1-2 minutes and observe appearance of red-pink ring (positive test).

#### • CATALASE TEST

Add 2 drops of H<sub>2</sub>O<sub>2</sub> in well **6-CAT**, wait for 1-2 minutes and observe appearance of gas bubbles (positive test).

#### • METHYL RED TEST

Add 2 drops of methyl red reagent in well **7-MR**, wait for 1-2 minutes and observe appearance of red colouration (positive test).

#### • NITRATE TEST

Add 1 drop of sulphanilic acid and 1 drop of naftilamine in well **17-NIT**, wait for 1-2 minutes and observe appearance of red orange colouration (positive test).

#### • VP TEST

Add 2 drops of alpha-naphtol and 1 drop of NaOH 40% in well **18-VP**, wait for 20 minutes and observe appearance of red-pink ring (positive test).

## RESULTS INTERPRETATION

At the end of the incubation, observe the change in colour of the wells and interpret the results using Table n°2 and form the 6-digit code following the instructions given to the paragraph **FORMATION OF THE NUMERICAL CODE**. Then use IDENTIFICATION TABLE (Table n°3) to identify the bacteria.

Table n°2

Well	BIOCHEMICAL IDENTIFICATION	Well colour	
		Positive reaction	Negative reaction
<b>1-ONPG</b>	ONPG hydrolysis	Yellow	Colourless
<b>2-UR</b>	Urea hydrolysis	Fucsia-Red	Yellow
<b>3- H<sub>2</sub>S</b>	Production of Sulfurate hydrogen	Black	Yellow
<b>4-IND</b>	Indole production	Pink Ring	Yellow
<b>5-RAF</b>	Raffinose fermentation	Yellow-Golden Yellow	Orange-Red
<b>6-CAT</b>	Catalase production	Presence of bubbles	Absence of bubbles
<b>7-MR</b>	Production of organic acid	Pink- Red	Yellow
<b>8-ESC</b>	Aesculine hydrolysis	Black	Yellow
<b>9-ABN</b>	Aminoacil-β-naftilamide hydrolysis	Yellow	Colorless
<b>10-GLU</b>	Glucose fermentation	Yellow-Golden Yellow	Orange-Red
<b>11-ARL</b>	Arabitole fermentation	Yellow-Golden Yellow	Orange-Red
<b>12-MAL</b>	Maltose fermentation	Yellow-Golden Yellow	Orange-Red
<b>13-RAM</b>	Rhamnose fermentation	Yellow- Golden Yellow	Orange-Red
<b>14-AMDM</b>	α-methyl-D-mannoside fermentation	Yellow- Golden Yellow	Orange-Red
<b>15-XYL</b>	Xylose fermentation	Yellow-Golden Yellow	Orange-Red
<b>16-MAN</b>	Mannitol fermentation	Yellow-Golden Yellow	Orange-Red
<b>17-NIT</b>	Nitrate and nitrite reduction	Orange-Red	Yellow
<b>18-VP</b>	Acetoine production	Pink Ring	Yellow

## FORMATION OF THE NUMERICAL CODE

- Tests are divided into 6 groups of three, each one is indicated with a positiveness value of 1,2,4.
  - Value 1: first positive test of each group (ONPG, IND, MR, GLU, RAM, MAN);
  - Value 2: second positive test of each group (UR, RAF, ESC, ARL, AMDM, NIT);
  - Value 4: third positive test of each group (H<sub>2</sub>S, CAT, ABN, MAL, XYL, VP);
  - Value 0: negative reaction of each test.
- By adding the number of positive reactions in each group, is obtained a code with five numbers that, using IDENTIFICATION TABLE, allows germ's identification.

	Group I			Group II			Group III			Group IV			Group V			Group VI		
Well	ONPG	UR	H <sub>2</sub> S	IND	RAF	CAT	MR	ESC	ABN	GLU	ARL	MAL	RAM	AMDM	XYL	MAN	NIT	VP
Positivity code	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
Results	-	-	-	-	-	+	+	+	-	+	+	+	+	+	-	-	-	+
Value addition	0			4			3			7			3			4		
CODE NUMBER: 043734																		
IDENTIFICATION : <i>Listeria monocytogenes</i>																		

## SCHEME OF BIOCHEMICAL REACTIONS

MICROORGANISM	ONPG	UR	H <sub>2</sub> S	IND	RAF	CAT	MR	ESC	ABN	GLU	ARL	MAL	RAM	AMDM	XIL	MAN	NIT	VP
<i>L. monocytogenes</i>	-	-	-	-	-	+	+	+	-	+	+	V	V	+	-	-	-	+
<i>L. innocua</i>	-	-	-	-	-	+	+	+	+	+	+	V	V	+	-	-	-	+
<i>L. ivanovii</i>	-	-	-	-	-	+	+	+	+	+	+	V	-	-	+	-	-	+
<i>L. seeligeri</i>	-	-	-	-	-	+	+	+	+	+	+	+	-	V	+	-	-	+
<i>L. welshimeri</i>	-	-	-	-	-	+	+	+	+	+	+	+	V	+	+	-	-	+
<i>L. grayi sub. grayi</i>	-	-	-	-	-	+	+	+	+	+	V	+	V	+	V	+	-	+
<i>L. grayi sub. murrayi</i>	-	-	-	-	-	+	+	+	+	+	+	+	V	+	V	+	+	+
<i>Jonesia denitrificans</i>	+	-	-	-	-	+	+	+	-	+	V	+	-	-	+	-	+	-

+ = Positive reaction

V= Variable reaction

- = Negative reaction

### IDENTIFICATION TABLE

Table n°4

CODE	IDENTIFICATION	BETA HAEMOLYSIS	CAMP TEST S	CAMP TEST R
043324	<i>Listeria monocytogenes</i>	+	+	-
043334	<i>Listeria monocytogenes</i>	+	+	-
043724	<i>Listeria monocytogenes</i>	+	+	-
043734	<i>Listeria monocytogenes</i>	+	+	-
047324	<i>Listeria innocua</i>	-	-	-
047334	<i>Listeria innocua</i>	-	-	-
047344	<i>Listeria ivanovii</i>	+	-	+
047525	<i>Listeria grayi subsp.grayi</i>	-		
047535	<i>Listeria grayi subsp.grayi</i>	-		
047565	<i>Listeria grayi subsp.grayi</i>	-		
047575	<i>Listeria grayi subsp.grayi</i>	-		
047724	<i>Listeria innocua</i>	-		
047725	<i>Listeria grayi subsp.grayi</i>	-		
047727	<i>Listeria grayi subsp.murrayi</i>			
047734	<i>Listeria innocua</i>	-		
047735	<i>Listeria grayi subsp.grayi</i>	-		
047737	<i>Listeria grayi subsp.murrayi</i>			
047744	<i>Listeria ivanovii</i>	+	-	+
047744	<i>Listeria seeligeri</i>	+	+	-
047764	<i>Listeria seeligeri</i>	+	+	-
047764	<i>Listeria welshimeri</i>	-	-	
047765	<i>Listeria grayi subsp.grayi</i>	-	-	
047767	<i>Listeria grayi subsp.murrayi</i>			
047774	<i>Listeria welshimeri</i>	-		
047775	<i>Listeria grayi subsp.grayi</i>	-		
047777	<i>Listeria grayi subsp.murrayi</i>			
143542	<i>Jonesia denitrificans</i>			
143742	<i>Jonesia denitrificans</i>			

## QUALITY CONTROL

Each batch of **LISTERIA SYSTEM 18R** is subjected to quality control using the following reference strains:

- *Listeria monocytogenes*.....ATCC 35152
- *Listeria innocua*..... ATCC 33090
- *Listeria grayi subsp. grayi*..... ATCC 25401
- *Listeria seeligeri*.....ATCC 35967
- *Listeria welshimeri*..... ATCC 35897
- *Listeria ivanovii*.....ATCC 19119

## FACTORS THAT MAY INVALIDATE THE RESULTS

- Mixed or contaminated cultures.
- Inadequate standardization of inoculum.
- The strain does not belong to *Listeria* genus.
- Use of expired systems and additional reagents.
- Incorrect application of the procedure.

## PRECAUTIONS

The product, **LISTERIA SYSTEM 18R**, is classified as hazardous under current legislation, see the safety datasheet for a correct use. **LISTERIA SYSTEM 18R** must be used in the laboratory by properly trained personnel, using approved aseptic and safety methods for handling pathogenic agents.

## CONSERVATION

Store at 2-8°C in the original packaging. Keep away from sources of heat and avoid excessive changes in temperature. In such conditions the product will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.










## DISPOSAL OF USED MATERIAL

After use, **LISTERIA-SYSTEM 18R** and material that has come into contact with the sample must be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.

## PRESENTATION

Product	Code	Kit
LISTERIA-SYSTEM 18 R	71640	20 test

## TABLE OF SYMBOLS

<u>SYMBOL</u>	<u>MEANING</u>	<u>SYMBOL</u>	<u>MEANING</u>
	Batch code		Do not reuse
	Manufacturer		Contains sufficient for <n> tests
	Catalogue number		Fragile, handle with care
	Use by		Caution, consult accompanying documents
	Temperature limitation		

## BIBLIOGRAFIA / BIBLIOGRAPHY

1. Mc Lannchlin : *The identification of Listeria species in International Journal of Food Microbiology* **38** (1997) 77- 81
2. Bailey and Scott's. *Diagnostic Microbiology*. 7<sup>th</sup> ed. C.V. (1986). Mosby Company.
3. Edwin H. Lenette. *Manual of Clinical Microbiology*. 4<sup>th</sup> ed. (1985). AMS Washington.



**LIOFILCHEM** *Bacteriology Products*

**LIOFILCHEM S.r.l.**

Via Scozia, Zona Ind.le - 64026, Roseto D.A. (TE) - ITALY

Tel +390858930745 Fax +390858930330 Website: [www.liofilchem.net](http://www.liofilchem.net) E-mail: [liofilchem@liofilchem.net](mailto:liofilchem@liofilchem.net)