

TRANSIA® PLATE *Listeria*

LI0691

Kit certified by NF Validation ref. TRA 02/6-11/95

Conditions for use

TRANSIA® PLATE *Listeria* is a kit for detection of the *Listeria* genus (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. grayi*) in dairy products, meat products, seafood products, vegetables, composite foods, and environmental samples.

This test has been certified by NF Validation for the analysis of any product intended for all human food products (by performing assays on a broad range of foods) and industrial production environmental samples.

Principle of the test

TRANSIA® PLATE *Listeria* is an ELISA test (Enzyme Linked Immuno Sorbent Assay) based on a two-step sandwich-type reaction. The solid support of the reaction is a microtitration plate with dividable microwell strips. Specific antibodies of flagellar antigens are grafted on the microtitration plate wells.

Equipment

Kit components

- 1 microplate with dividable microwell strips with 96 wells (12 microwell strips of 8 microwells)
- Negative control: (bacterial antigens not belonging to the *Listeria* genus), ready-to-use, 10 mL
- Positive control: *Listeria monocytogenes* antigens, ready-to-use, 8 mL
- Conjugate (contains peroxidase conjugated anti *Listeria* antibodies), ready-to-use, 16 mL
- Wash buffer, 20 X concentration, 60 mL
- Substrate (urea H₂O₂), ready-to-use, 57 mL
- Chromogen (TMB), ready-to-use, 57 mL
- Stop solution (H₂SO₄), ready-to-use, 57 mL
- 1 microplate cover

Equipment needed but not provided

For preparation of samples and reagents:

- Balance and weighing bowls
- Stomacher® or macerator
- Erlenmeyer flasks (500 mL) or Stomacher bags and Sterile tubes (20 mL) for the subculture
- Magnetic stir plate
- Incubator at 30°C ± 1 °C
- Vortex mixer
- 5-mL or 10-mL screw-cap tubes that can withstand temperatures over 100 °C
- Boiling water bath (95-100 °C)
- Test tube and 1-liter beaker



For preparation of the supplemental wash solution:

1-liter graduated flask
pH meter

For the immunoenzyme test:

Plastic wash bottle or microplate washer
Absorbent paper
Microplate reader (450 nm)
100-1,000 μ L micropipette
Eppendorf Multipipette® repeating dispenser and 5- and 2.5-mL tips
Optional: Gemini automated system

For confirmation of positive results:

Incubator at 37 °C \pm 1 °C

Reagents needed but not provided

For preparation of samples:

Distilled water.
Fraser Broth and its Demi-Fraser Supplement
(for the pre-enrichment)
Fraser Broth with its supplement added
(for the subculture)

For preparation of the supplemental wash solution:

If the volume is insufficient for automatic washers, the wash solution can be supplemented by mixing PBS and Tween (see Preparation of reagents).

PBS :	NaCl	7.650 g
	Na ₂ HPO ₄ , 2H ₂ O	0.724 g
	KH ₂ PO ₄	0.210 g
	H ₂ O	1 L
Tween 20 (SIGMA: Ref. P1379)		0.5 mL

For confirmation of positive results:

Selective agar for *Listeria* testing in food: Oxford, Palcam, or *Listeria* chromogen agar per Ottaviani & Agosti.
Miniaturized panels for biochemical identification of *Listeria* (e.g., Microbact 12L or API *Listeria*).

Storage conditions

The kit should be stored at a temperature of 5 °C \pm 3 °C.

Safety

Follow Good Laboratory Practices when using this kit. Wear safety clothing and avoid all skin contact with the reagents. Do not swallow. Safety sheets are available on request.

Test procedure

Preparation of reagents:

Important: Take the reagents out of their boxes. Place the reagents at room temperature (18-25 °C) for at least one hour before use.

Never mix the reagents from 2 kits of different lots.

Preparation of Fraser and Demi-Fraser Broths and isolation mediums:

Follow the manufacturer's instructions.

Dilution of the wash buffer:

Dilute the contents of the wash buffer bottle to 1:20 in distilled water. Homogenize, and fill the wash bottle provided for this purpose.

The buffer can be prepared in advance or during the first incubation period. Refer to the "Immunoenzyme test: step 4" paragraph.

Store the diluted wash buffer at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for no more than 3 months.

Preparation of the supplemental wash buffer:

In a 1-liter beaker, dissolve the components in about 800 mL of distilled water while stirring.

Make up one liter in a graduated flask and verify that the pH is equal to 7.2 ± 0.1 .

Transfer to a bottle and label. Store at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for no more than 3 months.

Preparation of the substrate-chromogen mixture:

Because this mixture is not stable, it must be prepared fresh (stability is 2 hours). The preparation should be made up during the first incubation. Refer to the "Immunoenzyme test: step 4" paragraph.

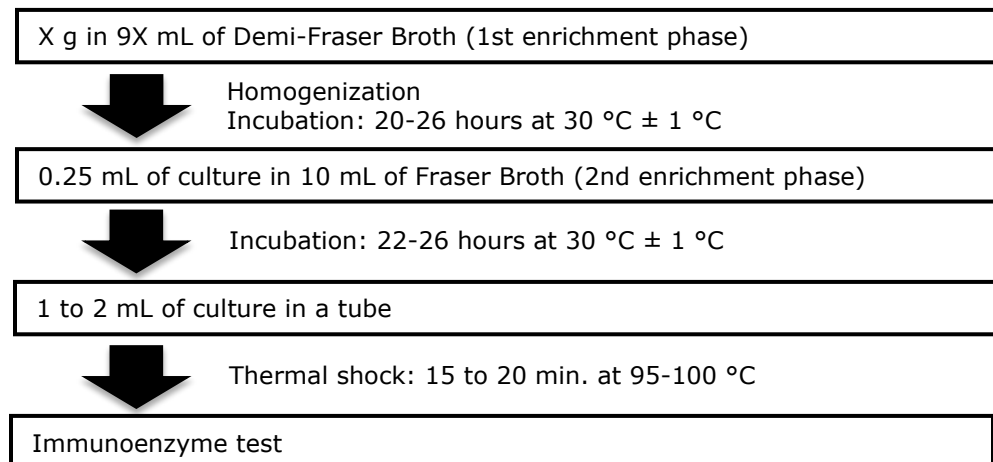
For each well used during the analysis, mix 60 μL of substrate with 60 μL of chromogen.

Preparation of Samples

1. Homogenize in the Stomacher, X grams (X mL) of sample with 9X mL of Demi-Fraser Broth with its Supplement added. For environmental samples, incubate a swab in 10 or 100 mL of Demi-Fraser Broth, depending on the size of the sample (keep the dilution factor at least 1:10). Test portions greater than 25 g have not been tested as part of the NF VALIDATION brand.
2. Incubate samples at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 20-26 hours.
3. After homogenization, inoculate 0.25 mL of culture in 10 mL of full Fraser Broth with its Supplement added.
4. Incubate at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 22 to 26 hours.
5. Heat 1 to 2 mL of Fraser Broth for 15 to 20 minutes in a boiling water bath. Cool to room temperature. Store the selective enrichment medium tubes for confirmation of positive ELISA results.

If the test cannot be done immediately after the 22 to 26 hours of Fraser Broth incubation, it can be stored for no more than 72 hours at $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ before heating, then ELISA test (the feasibility of storage was verified during the study done for NF VALIDATION certification).

Preparation of Samples



Test performance

Detection limit

The test detects 10^5 to 10^6 cells per mL in the broth after enrichment for 48 hours (including the pre-enrichment). The relative detection level estimated during the NF VALIDATION certification study was the same as that of the reference method. It was between 0.4 and 0.7 cells/25 grams for all categories combined.

Special applications

The TRANSIA® PLATE *Listeria* kit can be used on the Gemini automated system. For more information about using the kit on an automated system, refer to the instruction manual for the automated system being used.

Warning

The quality of the test result is directly related to the quality of the spectrophotometer reading. It is therefore essential that the reader be included in the laboratory's metrological inspection program and that it be inspected regularly.

Validations

NF VALIDATION: Any food product and environmental sample. Reference method: EN ISO 11290-1:2017. Validation attestation: TRA-02/6-11/95.

Note: The information contained in these instructions is based on our current knowledge and is intended to provide information about the use of this kit. Under no circumstances, however, does it constitute a guarantee of correct results in the case of a particular application.

Results interpretation

Test validation

The OD of the positive control (PC) should be greater than or equal to 0.70.

The OD of the negative control (NC) should be less than or equal to 0.30.

The test is valid only if the optical densities of the controls meet the above criteria.

Positivity threshold

The positivity threshold (PT) is obtained by adding 0.15 to the mean of the ODs of the negative controls:

$$PT = [(NC1 + NC2)/2] + 0.15$$

Positive samples

A sample is considered positive for *Listeria* if its OD is greater than or equal to the positivity threshold.

Negative samples

A sample is considered negative for *Listeria* if its OD is less than the positivity threshold.

Confirmation of positive results

All positive results must be confirmed as part of the NF VALIDATION brand by either:

1. Using traditional tests described in the methods standardized by CEN, ISO, or AFNOR (including the purification step). Isolate first from the selective medium used (Fraser) for the enrichment.
2. Isolation from the Fraser Broth on *Listeria* O&A agar (Ottaviani and Agosti, ALOA®). Typical colonies allow to confirm the positive result. If preferred, could be also confirmed with direct characterization on a biochemical identification panel of at least one suspicious colony isolated. If no colonies are isolated, perform a purification step first.
3. Isolation from the selective medium used (Fraser) on O&A agar. Typical colonies allow to confirm the positive result. If preferred, could be also confirmed with confirmation of at least one suspicious colony characteristic of *L. monocytogenes* on ALOA® Confirmation agar (confirmation of the *monocytogenes* species) or at least one suspicious colony that may or may not be characteristic of *L. monocytogenes* using Gram and catalase tests (confirmation of *Listeria* genus). Confirmation of the *Listeria* genus is mandatory if there is no *L. monocytogenes*.
4. Using any other NF VALIDATION certified method with a principle different from this method. The validated protocol of the second method must be followed in its entirety. This means that all steps prior to the intermediate step from which the confirmation starts should be common to both methods (for example, common enrichment with the same medium). The two validated methods (one used for detection and the other for confirmation) should therefore have a common trunk before branching off.

If the results do not agree (positive by TRANSIA® PLATE *Listeria* but not confirmed by one of the above options), the laboratory must use sufficient means to make sure that the rendered result is valid.

We then strongly recommend using additional isolation mediums using other biochemical characteristics of the *Listeria* genus.

Immunoenzyme test

Make sure that all reagents and samples are ready before use so that the addition of solutions to the microplate wells is not delayed, and make sure that they are at room temperature. Agitate each bottle manually or with the Vortex before use. The wash step is very important. When washing, direct a powerful stream toward the bottom of the wells. Verify that the cover is clean before use.



Samples + controls



Conjugated



1 Hour at room temperature



5 washes

Substrate/chromogen mixture



30 minutes at room temperature

Stop solution



1. Place the required number of microwell strips on the plate – 2 wells for the negative control, 1 well for the positive control, and 1 well per sample to be analyzed. Put the unused microwell strips back in the original packet containing the desiccator and seal it. Mark down the positions of the controls and samples on a worksheet.
2. Distribute 100 μL of controls and samples in the corresponding wells.
3. Distribute 100 μL of conjugated solution to each well using a repeating dispenser. Be careful not to touch the wells with the tip. Mix with a gentle circular motion. Put the cover on the plate.
4. Incubate at room temperature (18-25 $^{\circ}\text{C}$) for 1 hour. Prepare the wash solution (see § **Preparation of reagents**). Before the end of incubation, prepare the required volume of substrate/chromogen mixture (see **Preparation of reagents**).
5. Hold the plate firmly and empty the contents with a sharp wrist motion. Rinse each well, keeping the wash buffer in the wells for 5 to 10 seconds. Empty the microplate, then drain it on absorbent paper by rotating it and striking it firmly several times on the work surface. Perform this procedure at least 5 times.
6. Distribute 100 μL of the substrate/chromogen mixture into each well with a repeating dispenser. Discard unused solution.
Comment: The chromogen and the substrate can be added without prior mixing. Add 50 μL of substrate then 50 μL of chromogen to each well.
7. Allow to incubate at room temperature (18-25 $^{\circ}\text{C}$) for 30 minutes.
8. Add 50 μL of stop solution to each well following the same order used for adding the substrate/chromogen mixture. Mix the contents of the wells so the color changes completely. The blue turns to yellow.
9. Read the optical densities (OD) at 450 nm using a microplate reader (do the blank on air).

NF Validation certificate granted by AFNOR Certification for TRANSIA® PLATE Listeria as an alternative method of analysis for the detection of Listeria spp. in all food products and industrial production environmental samples in relation to the reference method described in the ISO EN 11290-1 international standard in accordance with EN ISO 16140-2 (2016). For more information about the end of validity of the NF VALIDATION certification, please refer to the certificate TRA 02/06-11/95 available on the website <http://nf-validation.afnor.org/en>.



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**ALTERNATIVE ANALYTICAL METHODS FOR
AGRIBUSINESS**

<http://nf-validation.afnor.org/en>

Manufacturing Entity

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