Probe4Monocytogenes®
Identification Kit for Listeria monocytogenes

1. INTENDED USE

Probe4Monocytogenes is a color nucleic acid mimic-based fluorescence in situ hybridization method (FISH) test intended for the identification of Listeria monocytogenes in fresh raw ground beef, fresh raw ground pork, butter lettuce, pasteurized cow’s milk and cooked shrimp.

2. SUMMARY AND EXPLANATION

Listeria monocytogenes is recognized worldwide as an important foodborne pathogen due to the high mortality rate (25 to 30%) associated with listeriosis.

Identification of L. monocytogenes is usually based on culture methods or molecular detection methods such as ELISA or PCR. Probe4Monocytogenes is a FISH method using peptide nucleic acid probes hybridizing to specific ribosomal RNA sequences of L. monocytogenes. The test provides rapid (within 3 hours) detection and identification of L. monocytogenes, after a 40 hour enrichment step.

3. PRINCIPLE OF THE PROCEDURE

A mixture of one Alexa Fluor 568-labeled probe for L. monocytogenes with an unlabeled blocker probe. After performing the fixation step with Fixation Solution 1 and Fixation Solution 2, hybridization occurs at 60 ± 1°C for 60 ± 5 min followed by a wash step with Wash Solution at 60 ± 1°C for 30 ± 5 min. Finally mounting medium is added and samples are examined by fluorescence microscopy.

4. COMPONENTS

- Fixation Solution 1: 4 mL of paraformaldehyde 4% (wt/vol) in phosphate buffered saline;
- Fixation Solution 2: 4 mL of ethanol 50% (vol/vol);
- Probe Solution: 4 mL of peptide nucleic acid probe in hybridization solution. Contains 5.5% (vol/vol) formamide;
- 60x Wash Solution: 50 mL of Tris-buffer with detergent;
- Mounting medium: 2 mL of immersion oil.

5. SAFETY PRECAUTIONS

For professional use only, by personnel trained and experienced in fluorescent microscopy.

The Probe Solution contains 5.5% (vol/vol) formamide with the following precautions and hazard concerns:

- Avoid inhalation of fumes.
- Wear appropriate personal protective equipment.
- If contact occurs with eyes, mouth, or skin, wash immediately with plenty of water.
- Avoid release to the environment.
- Do not use after the expiry date printed on the label.

6. STORAGE AND SHELF LIFE

Reagents should not be used after the expiry date printed on the labels. The kit should be stored at 5 ± 3°C when not in use. Place kit components at room temperature (20 ± 10°C) prior to use and return the kit components to 5 ± 3°C after use.

7. PREPARATION OF KIT COMPONENTS

Preparation of the Wash Solution

Prepare fresh Wash Solution by mixing 4 mL of 60x Wash Solution with 240 mL of distilled water.

Preparation of the Mounting Medium

The Mounting Medium should be left at room temperature (20 ± 10°C) for at least 5 min before use.

8. SPECIMEN PREPARATION

Samples preparation – Enrichment

- Food samples (solid or liquid form) should be handled similarly to sampling procedure found on ISO 11290-1:1996 Amd. 1:2004.
- Homogenize 25 g or mL of sample matrix in 225 mL of One Broth Listeria™ (Oxoid) in a stomacher bag for 60 ± 10 seconds.
- Incubate for 24 ± 2 h at 30 ± 1°C.
- Collect 1 mL of enriched solution and add to 9 mL of One Broth Listeria.
- Incubate for 16 ± 2 h at 30 ± 1°C.
- Collect 20 µL of the enriched sample and apply into a slide.
- Allow the slide to dry in an incubator at approximately 60°C for 10 ± 1 min.

9. PROCEDURE

Material required but not provided

- Coated slides with wells for epifluorescence microscopy
- Coverslips
- Coplin jars
- Closed container, protected from light, with moistened absorbent paper (e.g. petri dish wrapped with aluminium foil)
- Incubator (60 ± 1°C)
- Distilled water
- Fluorescence microscope equipped with a 60x or 100x oil objective and band pass filters FITC and TRITC

All steps are performed at room temperature (20 ± 10°C) unless otherwise stated. Gently mix the solutions of the kit before use. Prepare fresh Wash Solution for each run and proceed at follows:

Fixation:

- Add one drop of Fixation Solution 1 to the slide specimen and incubate for 10 ± 1 min.
- Remove the excess of Fixation Solution 1 by tilting the sample on absorbent paper.
- Add one drop of Fixation Solution 2 and incubate again for 10 ± 1 min.
- Remove excess of Fixation Solution 2 by tilting the sample on absorbent paper.
Hybridization:

- **IMPORTANT:** All steps following fixation MUST be performed in low light conditions as this may lead to fluorescence quenching. Turn the light off now before proceeding.
- Add one drop of Probe Solution to each well of the microscope slide with sample
- Add coverslip (avoiding air bubbles) and place the sample into a closed container, protected from the light, with a moistened paper towel inside.
- Incubate for 60 ± 5 min at 60 ± 1°C.
- **REMINDER:** Fill a coplin jar with previously prepared Wash Solution and let preheat also for 60 ± 5 min at 60 ± 1°C while the hybridization occurs.

Wash:

- **IMPORTANT:** keep performing the following steps in low light conditions.
- Remove carefully the coverslip and immerse slide in a coplin jar with preheated Wash Solution at 60 ± 1°C.
- Incubate for 30 ± 5 min at 60 ± 1°C.
- Remove the slide from the coplin jar and allow it to dry in an incubator at 60 ± 1°C.
- Add a drop of mounting medium, cover with a coverslip and add another drop of mounting medium on top of the coverslip.

The sample is ready for microscope observation. Note that the microscope observation must be carried out in low light conditions as well.

Interpretation of results

Examine specimen slides by fluorescence microscopy. *Listeria monocytogenes* will appear as bright red rod shaped cells in the red channel. A TRITC filter should be used for the visualization, to maximize the fluorescence signal.

Troubleshooting

False negative results with closed related strains may occur if the temperature is not accurately controlled during hybridization and washing. Positive and negative controls should be prepared. Positive controls consists preparing a *Listeria monocytogenes* CECT 938 suspension at approximately 10^7 CFU mL\(^{-1}\) and follow the assay procedure after applying 20 µL of the suspension into a slide. Negative controls consists of collecting 20 µL of a suspension at approximately 10^7 CFU mL\(^{-1}\) of *E. coli* ATCC 25922 and apply into a slide, and follow the procedure described above.

10. LIMITATIONS

The type and condition of the fluorescence microscope used will influence the visual appearance of the image obtained. The fluorescence intensity may vary due to the type of equipment use, the light source and the level of rRNA in the cells. Each laboratory should establish its own criteria for reading the results using appropriate controls.

11. PERFORMANCE CHARACTERISTICS

The Probe4Monocytogenes was evaluated in artificially contaminated food samples with 1 CFU per 25g of food sample with positive results. In Figure 1 it is possible observe a typical outcome obtained in meat samples.

![Probes](image)

**Fig. 1** – Probe4Monocytogenes outcome for ground beef samples inoculated with 1 CFU/25 g of food sample (A); and visualization of the same microscopic field at the green channel (B), where it is possible to observe autofluorescence of the food matrix.

12. DEFINITIONS

Consult the instructions for use
Contains sufficient for <N> tests

**REF**
Product code / catalogue number

GHS08 -- Danger

**LOT**
Batch code

Use by
Storage temperature limitations
Manufacturer

Final remarks

Please notice that Probe4Monocytogenes was optimized for the samples and procedure described in this leaflet. The kit may be used in other samples for research purposes. Contact us ([info@biomode-sa.com](mailto:info@biomode-sa.com)) for further information regarding the adaption of the standard protocol to your samples.