

**Certified Protocol Overview for
NF VALIDATION by AFNOR Certification
and
AOAC® PTMSM**

- BACGene *Salmonella* spp.
- BACGene *Listeria* spp.
- BACGene *Listeria monocytogenes*
- BACGene *Listeria* Multiplex



| | | BACGene <i>Salmonella</i> spp. - AFNOR Certified Protocols | | | | | | |
|---------------|-------------|--|------------------|-------------|------------------------|---|--|---|
| Sample Matrix | Media | Milk and dairy (25 g) | All foods (25 g) | Feed (25 g) | Pet food (up to 375 g) | Milk powders & infant formula (up to 375 g) | Milk powders & infant formula + probiotics (up to 375 g) | Environmentals |
| | Media | 1:10 BPW | | | 1:10 BPW Pre-warmed | | 1:10 BPW ² Pre-warmed | Swab (10 mL)/Sponge (100 mL)/wipe/cloth (225 mL)/Dust, process water (1:10) - BPW |
| Enrichment | T °C | 41,5 ± 1°C | | 37 ± 1°C | | | | |
| | Time | 18 - 24 h | 16 - 24 h | 18 - 24 h | | | | |
| Lysis | Prep | [Proteinase K] into Lysis Buffer I | | | | | | |
| | Dilute | 10 µL Enrichment + 90 µL Final Lysis Buffer I | | | | | | |
| | Time & T °C | 20 min @37 ± 2°C + 10 min @95 ± 2°C | | | | | | |
| PCR | Dilute | 5 µL Lysate on Ready-To-Use Plates | | | | | | |
| | Time | 100 Minutes | | | | | | |

| | | BACGene <i>Listeria</i> spp., <i>monocytogenes</i> & Multiplex - AFNOR Certified Protocols | | |
|---------------|-------------|--|--------------------------------|---|
| Sample Matrix | Media | All foods (25 g) | Dust & process water (25 g/mL) | Environmentals |
| | Media | 1:10 Actero™ Pre-Warmed | | Swab (10 mL)/Sponge (100 mL)/wipe/cloth (225 mL) Actero™ Pre-Warmed |
| Enrichment | T °C | 37 ± 1°C | | |
| | Time | 18 - 24 h | | |
| Lysis | Prep | [Proteinase K + Lysozyme] into Lysis Buffer L | | |
| | Dilute | 30 µL Enrichment + 70 µL Final Lysis Buffer L | | |
| | Time & T °C | 20 min @37 ± 2°C + 10 min @95 ± 2°C | | |
| PCR | Dilute | 5 µL Lysate on Ready-To-Use Plates | | |
| | Time | 100 Minutes | | |



BACGene Salmonella spp. - AOAC® Certified Protocols

| Sample Matrix | Frozen spinach (25 g) Pausteur. liquid eggs (25 g) Frozen cod fillet (25 g) | Pet food dry dog pellets (up to 375 g) | Ice cream with chocolate inclusions (25 g) | Dog pate (up to 375 g) | Marinated chicken with brine (up to 250 g) | Infant formula + probiotics (up to 375 g) | Raw whole milk (25 mL) | Ground beef (up to 375 g) Ground pork (up to 250 g) | Pork carcass cloth premoist with MRB | Pork carcass cloth premoist with diluents | Environmentals | Cocoa products (up to 375 g) | |
|--------------------|---|---|--|---------------------------|--|--|-------------------------------------|--|---|--|----------------|---|--|
| Media | 1:10 BPW | | | 1:10 BPW Pre-warmed | | | 1:10 BPW ² Pre-warmed | 1:10 BPW | 1:10 BPW Pre-warmed | 90 mL BPW Pre-Warmed | | Stainless steel (10 mL)/ Ceramic tiles (100 mL)/ process water (1:10) - BPW | 1:10 NF milk pre-warmed & Br. Green follow up with 1:50 BHI subbing |
| Enrichment T °C | 37 ± 1°C | | | | | | 41,5 ± 1°C | | | 37 ± 1°C | | | NF milk: 37 ± 1°C BHI: 41,5 ± 1°C |
| Time | 16 - 24 h | 18 - 24 h | | | 10 - 18 h | 18-24 h | | 10-18 h | | | | | NF milk: 18-24 h BHI: 3-5 h |
| Lysis Prep | [Proteinase K] into Lysis Buffer I | | | | | | | | | | | | |
| Dilute | 10 µL Enrichment + 90 µL Final Lysis Buffer I | | | | | | | | | | | NF milk: 18-24 h BHI: 3-5 h | |
| Time & T °C | 20 min @37 ± 2°C + 10 min @95 ± 2°C | | | | | | | | | | | | |
| PCR Dilute | 5 µL Lysate on Ready-To-Use Plates | | | | | | | | | | | | |
| Time | 100 Minutes | | | | | | | | | | | | |



BACGene Listeria spp., monocytogenes & Multiplex - AOAC® Certified Protocols

| Sample Matrix | Raw milk / smoked salmon / mayonnaise-based vegetable salad / frozen cantaloupe balls / Frankfurters / ice-cream with chocolate inclusions (all 25 g) | Soft white cheese & frozen shrimp (25 g) (only for Listeria spp.) | Process water (25 mL) | Environmental: Stainless steel (1"x1"), ceramic, plastic (4"x4") |
|--------------------|---|--|-----------------------|--|
| Media | 1:10 BPW | | | Stainless steel (10 mL) / ceramic (100 mL), plastic (1:10) - Actero™ Pre-warmed |
| Enrichment T °C | 37 ± 1°C | | | |
| Time | 18 - 24 h | | | |
| Lysis Prep | [Proteinase K + Lysozyme] into Lysis Buffer L | | | |
| Dilute | 30 µL Enrichment + 70 µL Final Lysis Buffer L | | | |
| Time & T °C | 20 min @37 ± 2°C + 10 min @95 ± 2°C | | | |
| PCR Dilute | 5 µL Lysate on Ready-To-Use Plates | | | |
| Time | 100 Minutes | | | |