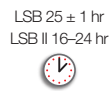
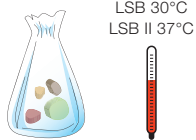




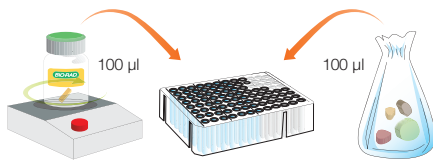
iQ-Check™ *Listeria monocytogenes* II, 3578124 iQ-Check *Listeria* spp., 3578113

Easy II Extraction Deep Well Protocol Using the Thermoshaker Quick Guide



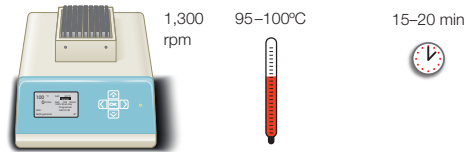
- Enrich the sample in *Listeria* Special Broth (LSB) (25 g in 225 ml; 125 g in 1,125 ml; environmental swab in 10 ml; environmental sponge in 60–225 ml) for 25 ± 1 hr at 30 ± 1°C
OR
- Enrich food products in LSB II (25 g in 225 ml; 125 g in 1,125 ml) for 18–24 hr at 37 ± 1°C
- Enrich environmental samples in LSB II (swab in 10 ml; sponge in 60–225 ml) for 16–24 hr at 37 ± 1°C

Check with your Bio-Rad™ representative for specific validations.

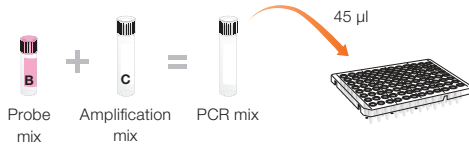


Be sure the lysis reagent is constantly stirring to keep it in suspension.

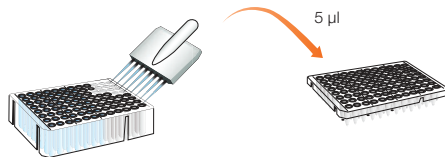
- Add 100 µl of the lysis reagent (reagent A and reagent F) to the deep well plate
- Transfer 100 µl of enriched sample to the deep well plate
Avoid shaking the sample bag to prevent collecting large fragments of food debris.
- Seal the deep well plate using the prepierced sealing film



- Incubate at 95–100°C for 15–20 min at 1,300 rpm in the thermoshaker
- Cool the deep well plate



- Prepare the PCR mix (see PCR Mix Calculation Guide on back)
- Distribute 45 µl/well in the PCR microplate



- Add 5 µl each of positive and negative controls to each well
- Transfer 5 µl of extracted DNA from the deep well plate to the PCR microplate
Do not vortex before collecting the sample.
Check that there are no bubbles.
- Seal the PCR microplate



- Launch CFX Manager Software, Industrial Diagnostic Edition (IDE)
- Create the PCR plate setup
- Start the amplification by clicking **Run**

* Steps automated by iQ-Check Prep System.

For detailed instructions, review the kit user guide.

PCR Mix Calculation Guide

To find the correct volumes to use when preparing the PCR mix, add the total number of samples and controls to be analyzed and find the corresponding volumes of reagent B and reagent C in the table.

| Total Number of Samples and Controls | Probes Reagent B, μ l | Amplification Mix Reagent C, μ l | Total Number of Samples and Controls | Probes Reagent B, μ l | Amplification Mix Reagent C, μ l | Total Number of Samples and Controls | Probes Reagent B, μ l | Amplification Mix Reagent C, μ l |
|--------------------------------------|---------------------------|--------------------------------------|--------------------------------------|---------------------------|--------------------------------------|--------------------------------------|---------------------------|--------------------------------------|
| 1 | 5 | 40 | 33 | 178 | 1,400 | 65 | 351 | 2,800 |
| 2 | 11 | 86 | 34 | 184 | 1,500 | 66 | 356 | 2,900 |
| 3 | 16 | 130 | 35 | 189 | 1,500 | 67 | 362 | 2,900 |
| 4 | 22 | 173 | 36 | 194 | 1,600 | 68 | 367 | 2,900 |
| 5 | 27 | 216 | 37 | 200 | 1,600 | 69 | 373 | 3,000 |
| 6 | 32 | 259 | 38 | 205 | 1,600 | 70 | 378 | 3,000 |
| 7 | 38 | 302 | 39 | 211 | 1,700 | 71 | 383 | 3,100 |
| 8 | 43 | 346 | 40 | 216 | 1,700 | 72 | 389 | 3,100 |
| 9 | 49 | 389 | 41 | 221 | 1,800 | 73 | 394 | 3,200 |
| 10 | 54 | 432 | 42 | 227 | 1,800 | 74 | 400 | 3,200 |
| 11 | 59 | 475 | 43 | 232 | 1,900 | 75 | 405 | 3,200 |
| 12 | 65 | 518 | 44 | 238 | 1,900 | 76 | 410 | 3,300 |
| 13 | 70 | 562 | 45 | 243 | 1,900 | 77 | 416 | 3,300 |
| 14 | 76 | 605 | 46 | 248 | 2,000 | 78 | 421 | 3,400 |
| 15 | 81 | 648 | 47 | 254 | 2,000 | 79 | 427 | 3,400 |
| 16 | 86 | 691 | 48 | 259 | 2,100 | 80 | 432 | 3,500 |
| 17 | 92 | 734 | 49 | 265 | 2,100 | 81 | 437 | 3,500 |
| 18 | 97 | 778 | 50 | 270 | 2,200 | 82 | 443 | 3,500 |
| 19 | 103 | 821 | 51 | 275 | 2,200 | 83 | 448 | 3,600 |
| 20 | 108 | 864 | 52 | 281 | 2,200 | 84 | 454 | 3,600 |
| 21 | 113 | 907 | 53 | 286 | 2,300 | 85 | 459 | 3,700 |
| 22 | 119 | 950 | 54 | 292 | 2,300 | 86 | 464 | 3,700 |
| 23 | 124 | 994 | 55 | 297 | 2,400 | 87 | 470 | 3,800 |
| 24 | 130 | 1,000 | 56 | 302 | 2,400 | 88 | 475 | 3,800 |
| 25 | 135 | 1,100 | 57 | 308 | 2,500 | 89 | 481 | 3,800 |
| 26 | 140 | 1,100 | 58 | 313 | 2,500 | 90 | 486 | 3,900 |
| 27 | 146 | 1,200 | 59 | 319 | 2,500 | 91 | 491 | 3,900 |
| 28 | 151 | 1,200 | 60 | 324 | 2,600 | 92 | 497 | 4,000 |
| 29 | 157 | 1,300 | 61 | 329 | 2,600 | 93 | 502 | 4,000 |
| 30 | 162 | 1,300 | 62 | 335 | 2,700 | 94 | 508 | 4,100 |
| 31 | 167 | 1,300 | 63 | 340 | 2,700 | 95 | 513 | 4,100 |
| 32 | 173 | 1,400 | 64 | 346 | 2,800 | 96 | 518 | 4,100 |

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