

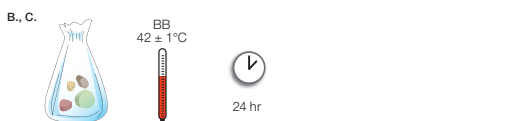
# iQ-Check *Campylobacter*, 3578135

## Easy I Extraction Deep Well Protocol Using the Thermoshaker



### A. Food samples

- Enrich the sample in supplemented Bolton Broth (25 g in 225 ml), 4 hr at 37°C
- Heat to 41.5°C for 20 hr under microaerobic conditions

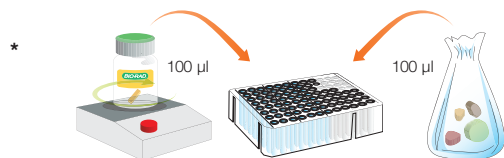


### B. Carcass rinses

- Rinse carcass in 400 ml buffered peptone water for 1 min. Remove 30 ml and add to 30 ml double strength, blood free Bolton Broth
- Enrich the sample, 24 hr at 42 ± 1°C under microaerobic conditions

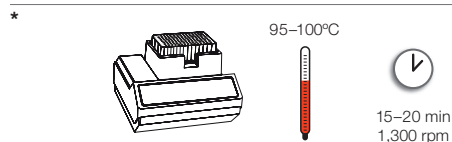
### C. Carcass sponges

- Enrich sponge in 25 ml double strength blood free Bolton Broth, 24 hr at 42 ± 1°C under microaerobic conditions

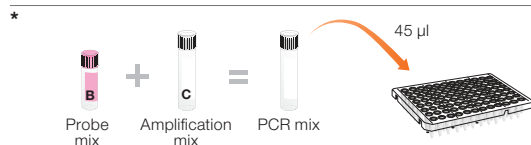


### Be sure the lysis reagent is constantly stirring to keep it in suspension.

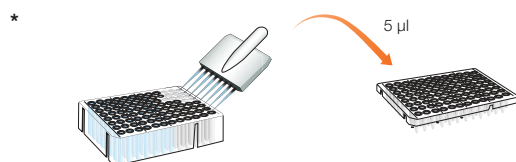
- Add 100 µl of the lysis reagent (reagent A) to each well of a deep well plate
- Transfer 100 µl of enriched sample to the deep well plate  
*Avoid shaking the stomacher bag to prevent collecting large fragments of food debris.*
- Seal the deep well plate using the prepierced sealing film



- 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 a 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 microplate  
**Do not vortex before collecting the sample.**  
*Check there are no bubbles.*
- Seal the PCR microplate



- Launch CFX Manager IDE Software
- Create the plate setup
- Start the amplification by clicking **Run**

\* Steps automated by iQ-Check Prep System.

For detailed instructions, review the kit instruction manual.

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# 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, µl | Amplification Mix Reagent C, µl | Total Number of Samples and Controls | Probes Reagent B, µl | Amplification Mix Reagent C, µl | Total Number of Samples and Controls | Probes Reagent B, µl | Amplification Mix Reagent C, µl |
|--------------------------------------|----------------------|---------------------------------|--------------------------------------|----------------------|---------------------------------|--------------------------------------|----------------------|---------------------------------|
| 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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