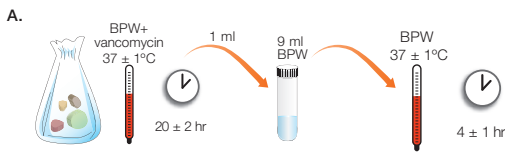


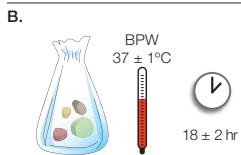
iQ-Check *Cronobacter* spp. 3578137

Easy I Extraction Deep Well Protocol Using the Thermoshaker



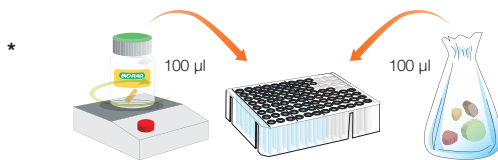
A. Infant formula samples

- Enrich the sample in buffered peptone water (BPW) supplemented with 10 µg/ml vancomycin (30 g in 270 ml) for 20 ± 2 hr at 37 ± 1°C
- Transfer 1 ml to 9 ml fresh BPW (without supplement) and incubate, 4 ± 1 hr at 37 ± 1°C



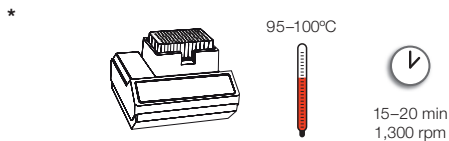
B. Environmental samples

- Enrich the sample in BPW (30 g in 270 ml) for 18 ± 2 hr at 37 ± 1°C

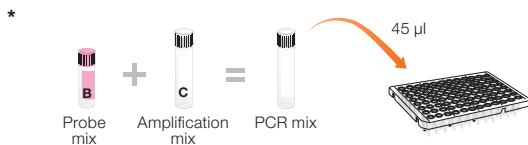


Be sure the lysis reagent is constantly stirring to keep it in suspension.

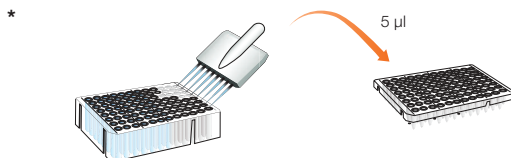
- Add 100 µl of the lysis reagent (reagent A) to each well of a deep well plate
- Transfer 100 µl of enriched sample to the deep well plate
Avoid shaking the stomacher bag to prevent collecting large fragments of food debris.
- Seal the deep well plate using the preperced sealing film



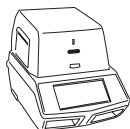
- Incubate at 95–100°C for 15–20 min at 1,300 rpm in the thermoshaker
- Cool the deep well plate



- 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.

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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