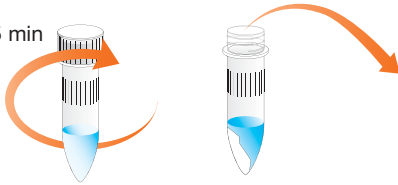
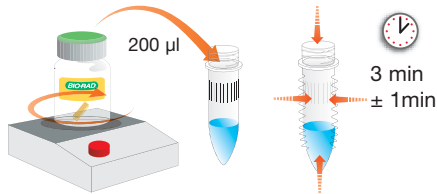


- Enrich the sample in pre-warmed buffered peptone water (25 g in 225 ml), 10 hr ± 2 hr at 37°C
- Collect 1 ml of enriched sample and place it in a 1.5 ml screwcap tube (*Shake stomacher bag to homogenize, and allow to decant before collecting. Avoid collecting large fragments of food debris*)

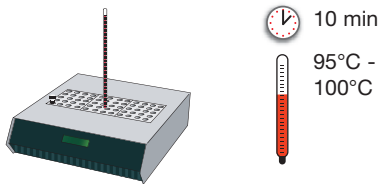
5 min



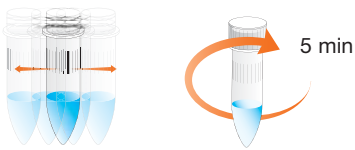
- Centrifuge at 10,000 - 12,000 g for 5 min
- Discard all the supernatant



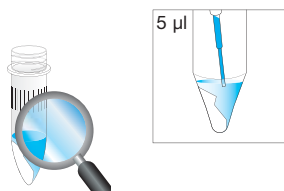
- Add 200 µl of the complete lysis reagent (reagent A + reagent F) to the pellet
- Lysis reagent must be constantly stirring in order to keep it in suspension
- Resuspend the pellet by pipetting the reagent up and down in the tube
- Grind at high speed for 3 min ± 1 min in vortex disruptor



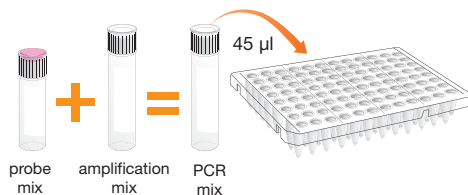
- Place the tube in the heating block
- Incubate at 95 - 100°C for 10 min



- Vortex at high speed
- Centrifuge at 10,000 - 12,000 g for 5 min



- Use 5 µl of the supernatant obtained for the amplification reaction
- Do not vortex before collecting 5 µl sample**



- Prepare the PCR mix (See PCR mix calculation guide)
- Distribute the PCR mix (45 µl)
- Distribute the samples and the controls (5 µl)
- Check that there are no bubbles
- Seal the microplate



- Set up software
- Create the plate setup
- Place the microplate into the thermocycler
- Start the amplification by clicking on "Run"

Please read the kit instruction manual and instrument user guide for complete and detailed instructions.

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 & controls	Probes - Reagent B (µl)	Amplification Mix Reagent C (µl)
1	5	40
2	11	86
3	16	130
4	22	173
5	27	216
6	32	259
7	38	302
8	43	346
9	49	389
10	54	432
11	59	475
12	65	518
13	70	562
14	76	605
15	81	648
16	86	691
17	92	734
18	97	778
19	103	821
20	108	864
21	113	907
22	119	950
23	124	994
24	130	1000
25	135	1100
26	140	1100
27	146	1200
28	151	1200
29	157	1300
30	162	1300
31	167	1300
32	173	1400
33	178	1400
34	184	1500
35	189	1500
36	194	1600
37	200	1600
38	205	1600
39	211	1700
40	216	1700
41	221	1800
42	227	1800
43	232	1900
44	238	1900
45	243	1900
46	248	2000
47	254	2000
48	259	2100

Total number of samples & controls	Probes - Reagent B (µl)	Amplification Mix Reagent C (µl)
49	265	2100
50	270	2200
51	275	2200
52	281	2200
53	286	2300
54	292	2300
55	297	2400
56	302	2400
57	308	2500
58	313	2500
59	319	2500
60	324	2600
61	329	2600
62	335	2700
63	340	2700
64	346	2800
65	351	2800
66	356	2900
67	362	2900
68	367	2900
69	373	3000
70	378	3000
71	383	3100
72	389	3100
73	394	3200
74	400	3200
75	405	3200
76	410	3300
77	416	3300
78	421	3400
79	427	3400
80	432	3500
81	437	3500
82	443	3500
83	448	3600
84	454	3600
85	459	3700
86	464	3700
87	470	3800
88	475	3800
89	481	3800
90	486	3900
91	491	3900
92	497	4000
93	502	4000
94	508	4100
95	513	4100
96	518	4100

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