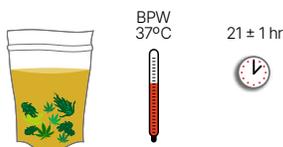




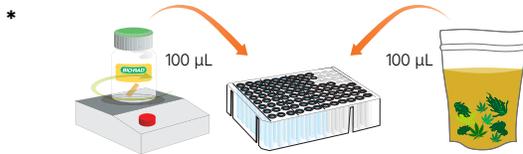
iQ-Check™ *E. coli* O157:H7, iQ-Check *Salmonella* II, and iQ-Check STEC VirX PCR Detection Kits

Harmonized Enrichment and Easy I Deep Well Extraction Protocol for Cannabis Quick Guide



Cannabis and cannabis-infused samples (Easy I)

- Enrich 1 g of the sample in 9 mL of prewarmed Buffered Peptone Water (BPW) for 21 ± 1 hr at $37 \pm 1^\circ\text{C}$
Check with your Bio-Rad representative for specific validations.

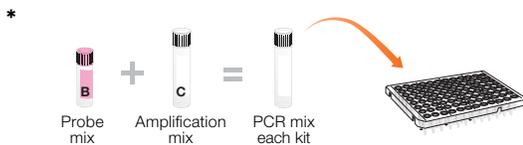


Be sure the lysis reagent is constantly stirring to keep it in suspension.

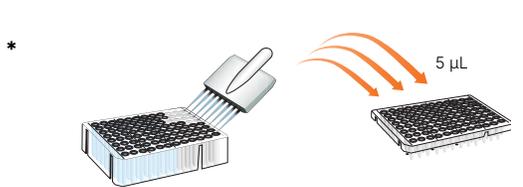
- Add 100 µL of the lysis reagent (Easy I: reagent A) to each well of a deep well plate
- Transfer 100 µL of enriched sample to the deep well plate
Avoid shaking the stomacher bag to prevent collecting large fragments of debris.
- Seal the deep well plate using the pre-pierced sealing film



- Incubate at $95\text{--}100^\circ\text{C}$ for 15–20 min at 1,300 rpm in the thermoshaker
- Cool the deep well plate



- Prepare the PCR mix for each organism/kit (see PCR Mix Calculation Guides)
- Distribute 20 µL/well of STEC PCR mix in a microplate
- Distribute 45 µL/well of *E.coli* O157:H7, *Salmonella* II PCR mix in a microplate



- For each kit, add 5 µL each of positive and negative controls to designated wells
- Transfer 5 µL/well of extracted DNA from the deep well plate to the PCR microplate for each target
It is recommended to dilute the DNA extract 1:10 in sterile water for inhibitory matrices such as cannabis flower/plant material and chocolate. Avoid pipetting beads. Do not vortex before collecting samples. Check there are no bubbles.
- Seal the PCR microplate



- Launch CFX Manager or CFX Maestro Software, Industrial Diagnostic Edition
- Create the plate setup
- Start the amplification by clicking **Run**

* Steps automated by iQ-Check Prep System.

For detailed instructions, review the kit instruction manuals.

PCR Mix Calculation Guide for the iQ-Check *E. coli* O157:H7 and *Salmonella* II Kits

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
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	1,000
25	135	1,100
26	140	1,100
27	146	1,200
28	151	1,200
29	157	1,300
30	162	1,300
31	167	1,300
32	173	1,400

Total Number of Samples and Controls	Probes Reagent B, μL	Amplification Mix Reagent C, μL
33	178	1,400
34	184	1,500
35	189	1,500
36	194	1,600
37	200	1,600
38	205	1,600
39	211	1,700
40	216	1,700
41	221	1,800
42	227	1,800
43	232	1,900
44	238	1,900
45	243	1,900
46	248	2,000
47	254	2,000
48	259	2,100
49	265	2,100
50	270	2,200
51	275	2,200
52	281	2,200
53	286	2,300
54	292	2,300
55	297	2,400
56	302	2,400
57	308	2,500
58	313	2,500
59	319	2,500
60	324	2,600
61	329	2,600
62	335	2,700
63	340	2,700
64	346	2,800

Total Number of Samples and Controls	Probes Reagent B, μL	Amplification Mix Reagent C, μL
65	351	2,800
66	356	2,900
67	362	2,900
68	367	2,900
69	373	3,000
70	378	3,000
71	383	3,100
72	389	3,100
73	394	3,200
74	400	3,200
75	405	3,200
76	410	3,300
77	416	3,300
78	421	3,400
79	427	3,400
80	432	3,500
81	437	3,500
82	443	3,500
83	448	3,600
84	454	3,600
85	459	3,700
86	464	3,700
87	470	3,800
88	475	3,800
89	481	3,800
90	486	3,900
91	491	3,900
92	497	4,000
93	502	4,000
94	508	4,100
95	513	4,100
96	518	4,100

PCR Mix Calculation Guide for the iQ-Check STEC VirX Kit

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	15	33	178	535	65	351	1,050
2	11	33	34	184	550	66	356	1,070
3	16	48	35	189	565	67	362	1,090
4	22	66	36	194	580	68	367	1,100
5	27	81	37	200	600	69	373	1,120
6	32	96	38	205	615	70	378	1,130
7	38	115	39	211	635	71	383	1,150
8	43	130	40	216	650	72	389	1,170
9	49	147	41	221	665	73	394	1,180
10	54	160	42	227	680	74	400	1,200
11	59	177	43	232	696	75	405	1,210
12	65	195	44	238	715	76	410	1,230
13	70	210	45	243	730	77	416	1,250
14	76	230	46	248	745	78	421	1,260
15	81	245	47	254	760	79	427	1,280
16	86	260	48	259	777	80	432	1,300
17	92	275	49	265	795	81	437	1,310
18	97	290	50	270	810	82	443	1,330
19	103	310	51	275	825	83	448	1,340
20	108	325	52	281	845	84	454	1,360
21	113	340	53	286	860	85	459	1,380
22	119	357	54	292	880	86	464	1,390
23	124	370	55	297	890	87	470	1,410
24	130	390	56	302	905	88	475	1,420
25	135	405	57	308	925	89	481	1,445
26	140	420	58	313	940	90	486	1,460
27	146	440	59	319	960	91	491	1,470
28	151	450	60	324	970	92	497	1,490
29	157	470	61	329	990	93	502	1,510
30	162	485	62	335	1,000	94	508	1,520
31	167	500	63	340	1,020	95	513	1,540
32	173	520	64	346	1,040	96	518	1,550

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