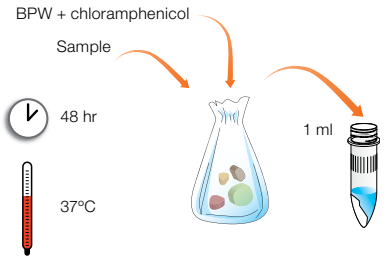
Quick Guide

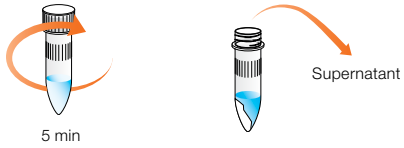
iQ-Check *Aspergillus*, 12010806

Standard II Extraction Protocol

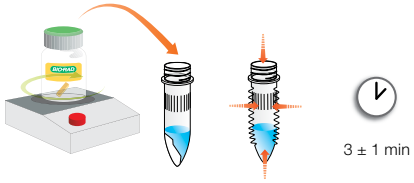


- Enrich the sample in room-temperature buffered peptone water (BPW) supplemented with 0.3 g/L chloramphenicol for 48 ± 3 hr at 37 ± 1°C
  - Cannabis and cannabis-infused products: 1:10 dilution of sample in BPW + chloramphenicol
  - Concentrates and cannabis-infused fats and oils: 1:10 dilution of sample in BPW + chloramphenicol with 1% v/v of Tergitol 7 (or equivalent surfactant)
- After enrichment, shake sample bag, then transfer 1 ml of enriched sample to a 1.5 ml screwcap vial

Note: If homogenization of samples using a 1:10 enrichment is difficult, a 1:30 enrichment may be used instead.

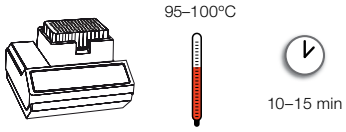


- Centrifuge at 10,000–12,000 x g for 5 min
- Discard all supernatant



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

- Add 200 µl of the lysis reagent (reagent A + reagent F) to the pellet
- Resuspend by pipetting up and down 5 times
- Grind at high speed for 3 ± 1 min in vortex disruptor



- Incubate tubes at 95–100°C for 10–15 min in the thermoshaker
- If a thermoshaker with tube adaptor is not available, transfer the entire sample volume to a deep well plate and incubate at 95–100°C for 10–15 min at 1,300–1,600 rpm

- For tubes, vortex at high speed, then centrifuge at 10,000–12,000 x g for 5 min
- For deep well plates, centrifuge at 2,250 x g for 2 min or allow to settle for 30 min undisturbed

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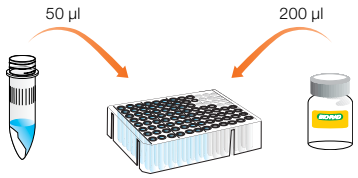
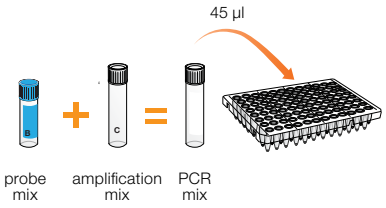


- For each sample, combine 50 µl of DNA extract with 200 µl of iQ-Check Purification Reagent in a new deep well plate. Mix by pipetting up and down 5 times

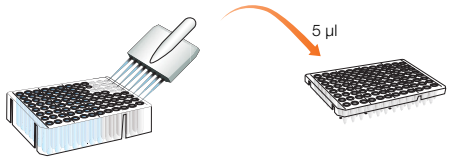
Note: Alternatively, dilute the DNA extract 1:10 in sterile water.

- Allow samples to settle for at least 5 min but not more than 1 hr at room temperature

Avoid pipetting beads. Do not vortex before collecting samples.



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

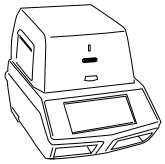


- Add 5 µl each of positive and negative controls
- Transfer 5 µl of extracted DNA from deep well plate to the microplate

Do not vortex before collecting the sample.

Eliminate any bubbles.

- Seal the microplate



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

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 anaylzed, and then 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

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