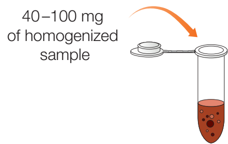
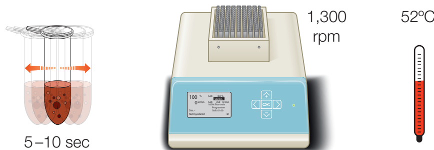
Invisorb Spin Tissue Mini Kit and ID-Check Speciation Kit

Quick Guide



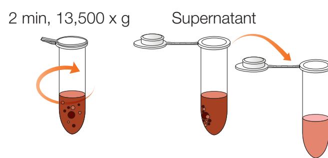
- Using a blender or similar equipment, homogenize a representative portion of coarsely chopped sample
- Weigh 40–100 mg of the homogenate or 200 µl of swab buffer into a 1.5 ml tube

Up to 200 mg of sample may be required for some matrices.

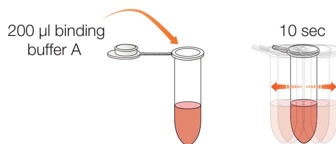


- Prepare reagents as described in the Invisorb Spin Tissue DNA Extraction Kit user guide. Begin to prewarm elution buffer to 52°C
- Add 400 µl lysis buffer G and 40 µl proteinase S and vortex thoroughly 5–10 sec
- Incubate the reaction tube at 52°C constantly shaking at 1,300 rpm until lysis is complete. For sample material that is difficult to lyse, vortex from time to time

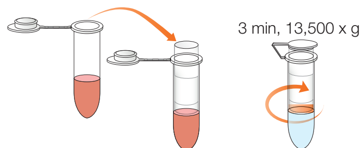
Processed, fatty, and highly seasoned foods may take longer. Lysis is complete when tube content is homogenous and sample is completely disrupted and no longer solid.



- Centrifuge at 13,500 x g for 2 min
- Transfer the supernatant into a new 1.5 ml tube (not provided)

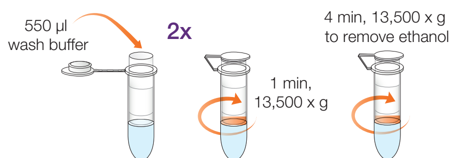


- Add 200 µl binding buffer A and vortex for 10 sec



- Place a spin filter in a 2.0 ml receiver tube. Transfer the reaction mixture into the spin filter and incubate for 1 min at room temperature
- Close the spin filter and centrifuge at 13,500 x g for 3 min
- Discard the filtrate and place the spin filter back into the receiver tube

Do not discard the spin filter at this step.



- Add 550 µl wash buffer, close the spin filter and centrifuge at 13,500 x g for 1 min. Discard the filtrate and place the spin filter back into the receiver tube
- Repeat the washing step one additional time. Discard the filtrate and put the spin filter back into the receiver tube. Remove the residual ethanol in the wash buffer by centrifugation for 4 min at 13,500 x g.
- For processed, fatty, and highly seasoned matrices that were difficult to lyse, an additional washing step is recommended.

Do not discard the spin filter at this step.

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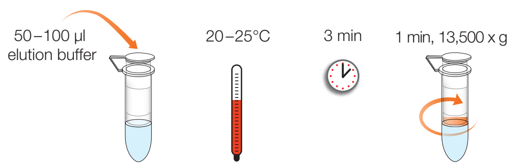
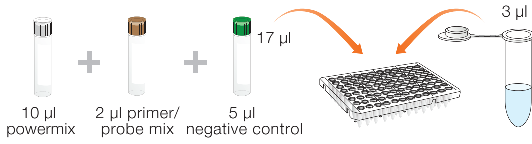


- Place the spin filter into a 1.5 ml receiver tube and add up to 100 µl of prewarmed (52°C) elution buffer. Incubate for 3 min at room temperature. Centrifuge for 1 min at 13,500 x g

Optional: *The DNA yield and purity can be assessed at this step by standardizing with a spectrophotometer or fluorometer.*

Do not discard the spin filter at this step.



- Prepare the PCR mix and distribute 17 µl to PCR plate or strips
- Remove spin filter and distribute 3 µl of extracted DNA from samples to PCR plate or strips
- Distribute 3 µl of negative and positive controls to PCR plate or strips
- Seal the PCR plate or strips

- Open CFX Manager Software, IDE, and create a User-defined Run Setup

Step	Temp, °C	Time	Cycles
Enzyme activation	95	5 min	1
Denaturing	95	20 sec	Repeat 30 times
Hybridization/extension/plate reading	60	1 min	

- Place PCR plate or strips into thermal cycler
- Start the amplification by clicking **Run**
- A result is considered positive when Cq ≤ 30

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 master mix, primer/probe mix, and negative control in the table.

Total Number of Samples and Controls	Powermix, µl	Primer/Probe Mix, µl	Negative Control, µl
1	10	2	5
2	30	6	15
3	40	8	20
4	50	10	25
5	60	12	30
6	70	14	35
7	80	16	40
8	90	18	45
9	100	20	50
10	110	22	55
11	120	24	60
12	130	26	65
13	150	30	75
14	160	32	80
15	170	34	85
16	180	36	90
17	190	38	95
18	200	40	100
19	210	42	105
20	220	44	110
21	230	46	115
22	240	48	120
23	250	50	125
24	260	52	130
25	270	54	135
26	290	58	145
27	300	60	150
28	310	62	155
29	320	64	160
30	330	66	165
31	340	68	170
32	350	70	175
33	360	72	180
34	370	74	185
35	380	76	190
36	390	78	195
37	400	80	200
38	420	84	210
39	430	86	215
40	440	88	220
41	450	90	225
42	460	92	230
43	470	94	235
44	480	96	240
45	490	98	245
46	500	100	250
47	510	102	255
48	520	104	260

Total Number of Samples and Controls	Powermix, µl	Primer/Probe Mix, µl	Negative Control, µl
49	530	106	265
50	540	108	270
51	560	112	280
52	570	114	285
53	580	116	290
54	590	118	295
55	600	120	300
56	605	122	305
57	620	124	310
58	630	126	315
59	640	128	320
60	650	130	325
61	660	132	330
62	670	134	335
63	690	138	345
64	700	140	350
65	710	142	355
66	720	144	360
67	730	146	365
68	740	148	370
69	750	150	375
70	760	152	380
71	770	154	385
72	780	156	390
73	790	158	395
74	800	160	400
75	810	162	405
76	830	166	415
77	840	168	420
78	850	170	425
79	860	172	430
80	870	174	435
81	880	176	440
82	890	178	445
83	900	180	450
84	910	182	455
85	920	184	460
86	930	186	465
87	940	188	470
88	960	192	480
89	970	194	485
90	980	196	490
91	990	198	495
92	1,000	200	500
93	1,010	202	505
94	1,020	204	510
95	1,030	206	515
96	1,040	208	520

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