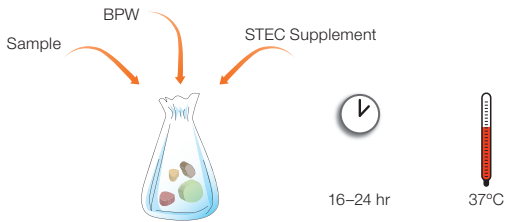
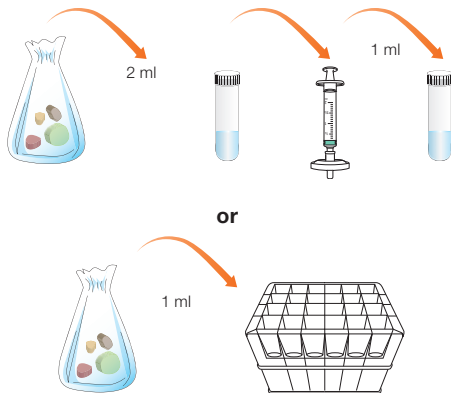


## dd-Check STEC Solution, 17004826

**Enrichment**

- Combine prewarmed (37°C) buffered peptone water (BPW), prepared STEC supplement, and sample into a filtered sample bag and homogenize
  - 25 g sample: 2.5 ml STEC supplement + 225 ml BPW
  - 200 g leafy greens: 6.5 ml STEC supplement + 450 ml BPW
  - 375 g sample: 15 ml STEC supplement + 1,125 ml BPW
- Enrich at 37°C for 16–24 hr
  - Do not shake the sample bag at the end of the enrichment.*

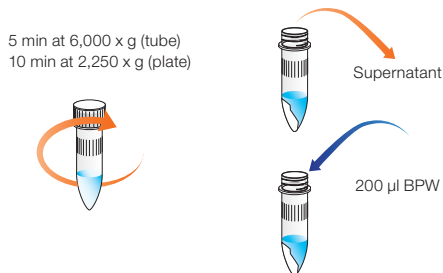
**Filtration****Tube Filtration Protocol**

- Collect 2 ml of enriched sample from the top of the bag and place it in a tube
- Filter enough sample into a new tube using a syringe and 10 µm filter to obtain 1 ml after filtration

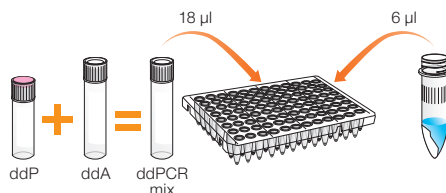
or

**Plate Filtration Protocol**

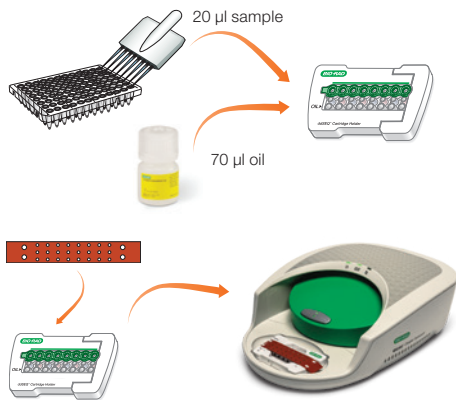
- Place a 24-well 10 µm filter plate above a 24-well collection plate and tighten with tape
- Add 1 ml of enriched sample to each well of the filtration plate and seal with film

**Centrifugation**

- Centrifuge tubes or plates
  - Tubes: 6,000 x g for 5 min
  - Plates: 2,250 x g for 10 min
- Discard the supernatant using care not to disrupt the pellet
- Add 200 µl of BPW (1 ml of BPW for flour matrices)
- Thoroughly resuspend the pellet by pipetting up and down

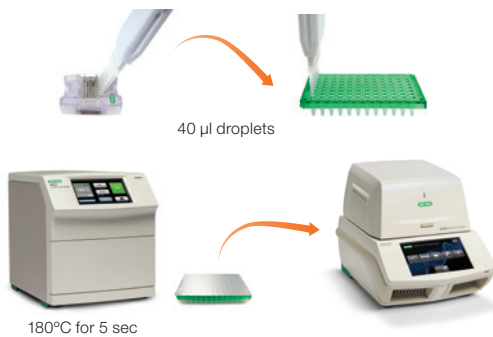
**PCR mix**

- Prepare PCR mix (see PCR Mix Calculation Guide)
- Distribute 18 µl of PCR mix in each well of a PCR plate
- Distribute 6 µl/well of sample or controls in the PCR plate



### Droplet Generation

- Carefully combine the PCR mix with the sample by pipetting up and down at least 5 times  
*Avoid creating bubbles.*
- Transfer 20 µl of each prepared sample to the sample wells (middle row) of the DG8 Cartridge  
*All eight wells must be filled.*
- Fill each oil well (bottom row) with 70 µl of Droplet Generation Oil
- Hook the gasket over the cartridge holder
- Generate droplets with the QX200 Droplet Generator  
*The droplet generation step must be performed within a maximum of 30 minutes.*



### PCR Amplification

- Slowly aspirate all of the droplets (40 µl) from the DG8 Cartridge using a Rainin pipet and tips
- Transfer into a 96-well PCR plate
- Seal the plate with foil using the PX1 Plate Sealer (180°C for 5 sec)
- Perform PCR amplification in the CFX96 Touch Deep Well PCR Detection System using the recommended dd-Check STEC protocol



### Reading

- Read the plate immediately after PCR amplification using the QX200 Droplet Reader and Qx IDE Manager Software

## PCR Mix Calculation Guide

To find the correct volumes to use when preparing the ddPCR mix, add the total number of samples and controls to be analyzed and find the corresponding volumes of reagent ddP and reagent ddA in the table.

Total Number of Samples and Controls	Primer-Probe Mix Reagent ddP, $\mu$ l	Amplification Mix Reagent ddA, $\mu$ l	Total Number of Samples and Controls	Primer-Probe Mix Reagent ddP, $\mu$ l	Amplification Mix Reagent ddA, $\mu$ l	Total Number of Samples and Controls	Primer-Probe Mix Reagent ddP, $\mu$ l	Amplification Mix Reagent ddA, $\mu$ l
1	12	6	33	430	215	65	840	420
2	26	13	34	450	225	66	850	425
3	40	20	35	460	230	67	870	435
4	52	26	36	470	235	68	880	440
5	66	33	37	490	245	69	890	445
6	80	40	38	500	250	70	910	455
7	92	46	39	510	255	71	920	460
8	104	52	40	520	260	72	930	465
9	120	60	41	540	270	73	940	470
10	130	65	42	550	275	74	960	480
11	146	73	43	560	280	75	970	485
12	160	80	44	570	285	76	990	495
13	170	85	45	580	290	77	1,000	500
14	180	90	46	590	295	78	1,000	500
15	200	100	47	610	305	79	1,000	500
16	210	105	48	620	310	80	1,050	525
17	220	110	49	630	315	81	1,050	525
18	230	115	50	650	325	82	1,050	525
19	250	125	51	660	330	83	1,050	525
20	260	130	52	670	335	84	1,100	550
21	280	140	53	680	340	85	1,100	550
22	290	145	54	700	350	86	1,100	550
23	300	150	55	710	355	87	1,100	550
24	320	160	56	720	360	88	1,150	575
25	330	165	57	740	370	89	1,150	575
26	340	170	58	750	375	90	1,150	575
27	350	175	59	770	385	91	1,150	575
28	370	185	60	780	390	92	1,200	600
29	380	190	61	790	395	93	1,200	600
30	390	195	62	800	400	94	1,200	600
31	410	205	63	820	410	95	1,200	600
32	420	210	64	830	415	96	1,250	625

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