Gradient Recipes

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Abstract

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Guidelines

1) 10-40% linear sucrose gradient for SW27 (or SW28) rotor tubes for 6 gradients

96.0 mL of sucrose (600 mg/mL)

48.0 mL of 3X buffer

144.0 mL total of 400 mg/mL sucrose

add 9.0 mL to each of 6 gradients (90.0 mL remaining)

90.0 mL of sucrose (400 mg/mL)

30.0 mL of 1X buffer

120.0 mL total of 300 mg/mL sucrose

add 9.0 mL to each of 6 gradients (66.0 mL remaining)

66.0 mL of sucrose (300 mg/mL)

33.0 mL of 1X buffer

99.0 mL total of 200 mg/mL sucrose

add 9.0 mL to each of 6 gradients (45.0 mL remaining)

45.0 mL of sucrose (200 mg/mL)

45.0 mL of 1X buffer

90.0 mL total of 100 mg/mL sucrose

add 6.0 mL to each of 6 gradients (discard the remainder)

2) 20-50% linear sucrose gradient for SW27 (or SW28) rotor tubes for six gradients

120.0 mL of sucrose (600 mg/mL)

24.0 mL of 6X buffer

144.0 mL total of 500 mg/mL sucrose

add 9.0 mL to each of 6 gradients (90.0 mL remaining)

90.0 mL of sucrose (500 mg/mL)

22.8 mL of 1X buffer

112.8 mL total of 400 mg/mL sucrose

add 9.0 mL to each of 6 gradients (58.8 mL remaining)

58.8 mL of sucrose (400 mg/mL)

19.8 mL of 1X buffer

78.6 mL total of 300 mg/mL sucrose

add 9.0 mL to each of 6 gradients (24.6 mL remaining)

24.6 mL of sucrose (300 mg/mL)

12.6 mL of 1X buffer

37.2 mL total of 200 mg/mL sucrose

add 6.0 mL to each of 6 gradients (discard the remainder)

3) 20-40% linear sucrose gradient for SW27 (or SW28) rotor tubes for 6 gradients

96.0 mL of sucrose (600 mg/mL)

48.0 mL of 3X buffer

144.0 mL total of 400 mg/mL sucrose

add 10.0 mL to each of 6 gradients (84.0 mL remaining)

84.0 mL of sucrose (400 mg/mL)

28.0 mL of 1X buffer

112.0 mL total of 300 mg/mL sucrose

add 10.0 mL to each of 6 gradients (52.0 mL remaining)

52.0 mL of sucrose (300 mg/mL)

26.0 mL of 1X buffer

78.0 mL total of 200 mg/mL sucrose

add 10.0 mL to each of 6 gradients (discard the remainder)

4) 5-20% linear sucrose gradient for SW27 (or SW28) rotor tubes for 6 gradients

48.0 mL of sucrose (600 mg/mL)

96.0 mL of 1.5X buffer

144.0 mL total of 200 mg/mL sucrose

add 9.0 mL to each of 6 gradients (90.0 mL remaining)

90.0 mL of sucrose (200 mg/mL)

30.0 mL of 1X buffer

120.0 mL total of 150 mg/mL sucrose

add 9.0 mL to each of 6 gradients (66.0 mL remaining)

66.0 mL of sucrose (150 mg/mL)

33.0 mL of 1X buffer

99.0 mL total of 100 mg/mL sucrose

add 9.0 mL to each of 6 gradients (45.0 mL remaining)

45.0 mL of sucrose (100 mg/mL)

45.0 mL of 1X buffer

90.0 mL total of 50 mg/ml sucrose

add 6.0 mL to each of 6 gradients (discard the remainder)

5) 10-40% linear sucrose gradient for SW41 rotor tubes for 6 gradients

36.0 mL of sucrose (600 mg/mL)

18.0 mL of 3X buffer

54.0 mL total of 400 mg/mL sucrose

add 3.5 mL to each of 6 gradients (33.0 mL remaining)

33.0 mL of sucrose (400 mg/mL)

11.0 mL of 1X buffer

44.0 mL total of 300 mg/mL sucrose

add 3.5 mL to each of 6 gradients (23.0 mL remaining)

23.0 mL of sucrose (300 mg/mL)

11.5 mL of 1X buffer

34.5 mL total of 200 mg/mL sucrose

add 3.5 mL to each of 6 gradients (13.5 mL remaining)

13.5 mL of sucrose (200 mg/mL)

13.5 mL of 1X buffer

27.0 mL total of 100 mg/mL sucrose

add 1.5 mL to each of 6 gradients (discard the remainder)

6) 15-30% linear sucrose gradint for SW41 rotor tubes for 6 gradients

36.0 mL of sucrose (600 mg/mL)

36.0 mL of 2X buffer

72.0 mL total of 300 mg/mL sucrose

add 3.5 mL to each of 6 gradients (51.0 mL remaining)

51.0 mL of sucrose (300 mg/mL)

10.2 mL of 1X buffer

61.2 mL total of 250 mg/mL sucrose

add 3.5 mL to each of 6 gradients (40.0 mL remaining)

40.0 mL of sucrose (250 mg/mL)

10.0 mL of 1X buffer

50.0 mL total of 200 mg/mL sucrose

add 3.5 mL to each of 6 gradients (29.0 mL remaining)

29.0 mL of sucrose (200 mg/mL)

10.0 mL of 1X buffer

39.0 mL total of 150 mg/mL sucrose

add 1.5 mL to each of 6 gradients (discard the remainder)

7) 5-20% linear sucrose gradient for SW41 rotor tubes for 6 gradients

18.0 mL of sucrose (600 mg/mL)

36.0 mL of 1.5X buffer

54.0 mL total of 200 mg/mL sucrose

add 3.5 mL to each of 6 gradients (33.0 mL remaining)

33.0 mL of sucrose (200 mg/mL)

11.0 mL of 1X buffer

44.0 mL total of 150 mg/mL sucrose

add 3.5 mL to each of 6 gradients (23.0 mL remaining)

23.0 mL of sucrose (150 mg/mL)

11.5 mL of 1X buffer

34.5 mL total of 100 mg/mL sucrose

add 3.5 mL to each of 6 gradients (13.5 mL remaining)

13.5 mL of sucrose (100 mg/mL)

13.5 mL of 1X buffer

27.0 mL total of 50 mg/mL sucrose

add 1.5 mL to each of 6 gradients (discard the remainder)

8) 5-20% linear sucrose gradient for SW50.1 tubes for 6 gradients

8.0 mL of sucrose (600 mg/mL)

16.0 mL of 1.5X buffer

24.0 mL total of 200 mg/mL sucrose

add 1.2 mL to each of 6 gradients (16.8 mL remaining)

16.8 mL of sucrose (200 mg/mL)

5.6 mL of 1X buffer

22.4 mL total of 150 mg/mL sucrose

add 1.2 mL to each of 6 gradients (15.2 mL remaining)

15.2 mL of sucrose (150 mg/mL)

7.6 mL of 1X buffer

22.8 mL total of 100 mg/mL sucrose

add 1.2 mL to each of 6 gradients (15.6 mL remaining)

15.6 mL of sucrose (100 mg/mL)

15.6 mL of 1X buffer

31.2 mL total of 50 mg/mL sucrose

add 1.2 mL to each of 6 gradients (discard the remainder)

9) 10-40% linear sucrose gradient for SW50.1 rotor tubes for 6 gradients

16.0 mL of sucrose (600 mg/mL)

8.0 mL of 3X buffer

24.0 mL total of 400 mg/mL sucrose

add 1.2 mL to each of 6 gradients (16.8 mL remaining)

16.8 mL of sucrose (400 mg/mL)

5.6 mL of 1X buffer

22.4 mL total of 300 mg/mL sucrose

add 1.2 mL to each of 6 gradients (15.2 mL remaining)

15.2 mL of sucrose (300 mg/mL)

7.6 mL of 1X buffer

22.8 mL total of 200 mg/mL sucrose

add 1.2 mL to each of 6 gradients (15.6 mL remaining)

15.6 mL of sucrose (200 mg/mL)

15.6 mL of 1X buffer

31.2 mL total of 100 mg/mL sucrose

add 1.2 mL to each of 6 gradients (discard the remainder)

10) 10-40% linear sucrose gradient for SW60 rotor tubes for 6 gradients

12.0 mL of sucrose (600 mg/mL)

6.0 mL of 3X buffer

18.0 mL total of 400 mg/mL sucrose

add 1.0 mL to each of 6 gradients (12.0 mL remaining)

12.0 mL of sucrose (400 mg/mL)

4.0 mL of 1X buffer

16.0 mL total of 300 mg/mL sucrose

add 1.0 mL to each of 6 gradients (10.0 mL remaining)

10.0 mL of sucrose (300 mg/mL)

5.0 mL of 1X buffer

15.0 mL total of 200 mg/mL sucrose

add 1.0 mL to each of 6 gradients (9.0 mL remaining)

9.0 mL of sucrose (200 mg/mL0

9.0 mL of 1X buffer

18.0 mL total of 100 mg/mL sucrose

add 1.0 mL to each of 6 gradients (discard the remainder)

11) 5-25% linear sucrose gradient for SW41 rotor tubes for 6 gradients

25.0 mL of sucrose (600 mg/mL)

5.0 mL of d-H_2O

30.0 mL total of sucrose (500 mg/mL)

23.8 mL of sucrose (500 mg/mL)

23.8 mL of 2X buffer

47.6 mL total of 250 mg/mL sucrose

add 2.4 mL to each of 6 gradients (33.2 mL remaining)

33.2 mL of sucrose (250 mg/mL)

8.3 mL of 1X buffer

41.5 mL total of 200 mg/mL sucrose

add 2.4 mL to each of 6 gradients (27.1 mL remaining)

27.1 mL of sucrose (200 mg/mL)

9.0 mL of 1X buffer

36.1 mL total of 150 mg/mL sucrose

add 2.4 mL to each of 6 gradients (21.7 mL remaining)

21.7 mL of sucrose (150 mg/mL)

10.8 mL of 1X buffer

32.5 mL total of 100 mg/mL sucrose

add 2.4 mL to each of 6 gradients (18.1 mL remaining)

18.1 mL of sucrose (100 mg/mL)

18.1 mL of 1X buffer

36.2 mL total of 50 mg/mL sucrose

add 2.0 mL to each of 6 gradients (discard the remainder)

12) 5-25% linear sucrose gradient for SW50.1 rotor tubes for 6 gradients

10.0 mL of sucrose (600 mg/mL)

2.0 mL of d-H_2O

12.0 mL total of 500 mg/mL sucrose

11.0 mL of sucrose (500 mg/mL)

11.0 mL of 2X buffer

22.0 mL total of 250 mg/mL sucrose

add 1.2 mL to each of 6 gradients (14.8 mL remaining)

14.8 mL of sucrose (250 mg/mL)

4.7 mL of 1X buffer

19.5 mL total of 190 mg/mL sucrose

add 1.2 mL to each of 6 gradients (12.3 mL remaining)

12.3 mL of sucrose (190 mg/mL)

7.2 mL of 1X buffer

19.5 mL total of 120 mg/mL sucrose

add 1.2 mL to each of 6 gradients (12.3 mL remaining)

12.3 mL of sucrose (120 mg/mL)

17.2 mL of 1X buffer

29.5 mL total of 50 mg/mL sucrose

add 1.2 mL to each of 6 gradients (discard the remainder)

13) 5-25% linear sucrose gradient for SW60 tubes for 6 gradients

10.0 mL of sucrose (600 mg/mL)

2.0 mL of d-H_2O

12.0 mL total of 500 mg/mL sucrose

11.0 mL of sucrose (500 mg/mL)

11.0 mL of 2X buffer

22.0 mL total of 250 mg/mL sucrose

add 1.2 mL to each of 6 gradients (14.8 mL remaining)

14.8 mL of sucrose (250 mg/mL)

4.7 mL of 1X buffer

19.5 mL total of 190 mg/mL sucrose

add 1.2 mL to each of 6 gradients (12.3 mL remaining)

12.3 mL of sucrose (190 mg/mL)

7.2 mL of 1X buffer

19.5 mL total of 120 mg/mL sucrose

add 1.2 mL to each of 6 gradients (12.3 mL remaining)

12.3 mL of sucrose (120 mg/mL)

17.2 mL of 1X buffer

29.5 mL total of 50 mg/mL sucrose

add 1.0 mL to each of 6 gradients (discard the remainder)

14) Linear log sucrose gradient for SW27 (or SW28) rotor tubes, 14°C, Pp=1.30 for 6 gradients

Tube	Sucrose Layer	Sucrose (600 mg/ml)	2X buffer	d-H_2O	Total
	Conc.	*(mL)*	*(mL)*	*(mL)*	*Volume*
1	334 mg	3.0	12.23	9.77	0.0	22.0
2	304 mg	8.4	27.85	27.15	0.0	55.0
3	270 mg	7.8	22.50	22.50	2.5	50.0
4	230 mg	7.0	17.25	18.00	5.0	45.0
5	184 mg	5.5	11.04	12.00	7.0	36.0
6	120 mg	3.5	4.80	12.00	7.2	24.0
7	0 mg	3.0	0.00	11.00	11.0	22.0
		38.2			

15) Linear log sucrose gradient for SW41 rotor tubes, 14°C, Pp=1.50 for 6 gradients

Tube	Layer	Sucrose (600 mg/ml)	4X buffer	2X buffer	d-H_2O	Total
	(mL)	*(mL)*	*(mL)*	*(mL)*	*(mL)*	*Volume*
1	1.8	6.50	3.0	---	2.50	12.0
2	3.3	10.80	---	12.0	1.20	24.0
3	2.2	5.25	---	7.5	2.25	15.0
4	1.5	2.65	---	5.0	2.35	10.0
5	1.2	1.40	---	4.2	2.80	8.4
6	1.4	0.00	---	5.0	5.00	10.0
	11.1					

16) Linear log sucrose gradient for SW41 rotor tubes, 14°C, Pp=1.30 for 6 gradients

Tube	Layer	Sucrose (600 mg/ml)	2X buffer	d-H_2O	Total
	(mL)	*(mL)*	*(mL)*	*(mL)*	*Volume*
1	1.5	6.0	6.0	---	12.0
2	3.2	10.4	12.0	1.6	24.0
3	2.7	7.0	10.0	3.0	20.0
4	1.8	4.0	7.5	3.5	15.0
5	1.3	1.7	5.0	3.3	10.0
6	1.1	0.0	5.0	5.0	10.0
	11.6				

17) Linear log sucrose gradient for SW50.1 rotor tubes, 14°C, Pp=1.50 for 6 gradients

Tube	Layer	Sucrose (600 mg/ml)	4X buffer	2X buffer	d-H_2O	Total
	(mL)	*(mL)*	*(mL)*	*(mL)*	*(mL)*	*Volume*
1	0.92	5.40	2.5	---	2.10	10.0
2	1.66	6.75	---	7.5	0.75	15.0
3	1.15	5.25	---	7.5	2.25	15.0
4	0.65	2.15	---	4.0	1.85	8.0
5	0.55	1.60	---	4.0	2.40	8.0
6	0.37	0.00	---	3.0	3.00	6.0
	5.30					

18) Linear log sucrose gradient for SW60 rotor tubes, 14°C, Pp=1.50 for 6 gradients

Tube	Layer	Sucrose (600 mg/ml)	4X buffer	2X buffer	d-H_2O	Total
	(mL)	*(mL)*	*(mL)*	*(mL)*	*(mL)*	*Volume*
1	0.68	6.5	3.0	---	2.5	12.0
2	1.41	5.4	---	6.0	0.6	12.0
3	0.91	4.2	---	6.0	1.8	12.0
4	0.67	3.2	---	6.0	2.8	12.0
5	0.44	1.6	---	6.0	4.4	12.0
6	0.29	0.0	---	6.0	6.0	12.0
	4.40					

19) Linear log sucrose gradient for SW50.1 rotor tubes, 14°C, Pp=1.50 for 6 gradients

Tube	Layer	Sucrose (600 mg/ml)	2X buffer	1X buffer	Total
	(mL)	*(mL)*	*(mL)*	*(mL)*	*Volume*
1	1.10	4.93	4.93	0.14	10.0
2	1.80	5.50	5.50	4.00	15.0
3	1.08	2.83	2.83	4.34	10.0
4	0.72	1.50	1.50	7.00	10.0
5	0.60	0.00	0.00	5.00	5.0
	5.30				

20) 20-40% (w/w) linear CsCl gradient for SW60 rotor tubes for 6 gradients for virus

Tube	Percent	CsCl	1X buffer	Layer
		(gm)	*(mL)*	*(mL)*
1	40.0	5.0	7.5	1.1
2	33.5	4.0	7.9	1.1
3	26.5	3.0	8.3	1.1
4	20.0	2.0	8.0	1.0
				4.3

Centrifuge at 40,000 rpm, 4 hours, 4°C

21) 20-50% (w/w) linear CsCl gradients for SW60 rotor tubes for 6 gradients for virus

Tube	Percent	CsCl	1X buffer	Layer
		(gm)	*(mL)*	*(mL)*
1	50.0	7.5	7.5	1.1
2	40.0	5.0	7.5	1.1
3	30.0	3.0	7.0	1.1
4	20.0	2.0	8.0	1.0
				4.3

Centrifuge at 40,000 rpm, 18 hours, 25°C

22) 30-60% (w/w) linear CsCl gradient for SW60 rotor tubes for 6 gradients for DNA

Tube	Percent CsCl	1X buffer	Hoechst dye	Layer
		(gm)	*(mL)*	*#33258 (µL)*	*(mL)*
1	60.0	9.0	6.0	60.0	1.1
2	50.0	7.5	7.5	75.0	1.05
3	40.0	5.0	7.5	75.0	1.05
4	30.0	3.0	7.0	70.0	1.2
					4.4

Centrifuge at 35,000 rpm, 18 hours, 25°C

23) 45-60% (w/w) linear CsCl gradient for SW60 rotor tubes for 6 gradients for DNA

Tube	Percent CsCl	1X buffer	Hoechst dye	Layer
		(gm)	*(mL)*	*#33258 (µL)*	*(mL)*
1	60.0	9.0	6.0	60.0	1.1
2	55.0	9.0	7.4	74.0	1.05
3	50.0	7.5	7.5	75.0	1.05
4	45.0	6.0	7.3	73.0	1.2
					4.4

Centrifuge at 30,000 rpm, 24 hours, 25°C

24) 17-35% (w/w) linear Cs_2SO_4 gradients for SW60 rotor tubes for 6 gradients for virus

Tube	Percent Cs_2SO_4	1X buffer	Layer
		(gm)	*(mL)*	*(mL)*
1	35.0	4.2	7.8	1.1
2	29.0	3.2	7.8	1.1
3	23.0	2.3	7.7	1.1
4	17.0	1.7	8.3	1.0
				4.3

Centrifuge at 40,000 rpm, 18 hours, 25°C

Protocol