Alfa Wassermann's Unique Ultracentrifuge Designs

Enable Linear Process Scaling

Analysis of product peaks for each run showed similar peak height and width in both the KII large-scale, PKII Pilot-scale and the AW Promatix 1000™ scale ultracentrifuge systems. The peak density was similar in all centrifuge runs.

The figures describe a similar gradient shape across the Alfa Wassermann range of ultracentrifuges. From the figures both scalability and linearity of the particle separations was achieved.

Scalability was demonstrated because the run parameters remained the same, even though rotor assembly volume varied.

Linearity was confirmed: equivalent product separation and gradient formation were achieved in the KII large-scale, PKII Pilot-scale and the AW Promatix 1000™ scale. Product separation at the iso-dense layer and equivalent product peak shape in the gradient for each scale rotor assembly were achieved.

The gradient remains identical throughout the volumetric differences between each separation. The Alfa Wassermann ultracentrifuges retain the same separation parameters in a process in which the volume of the product sample centrifuged is to be scaled up or down.

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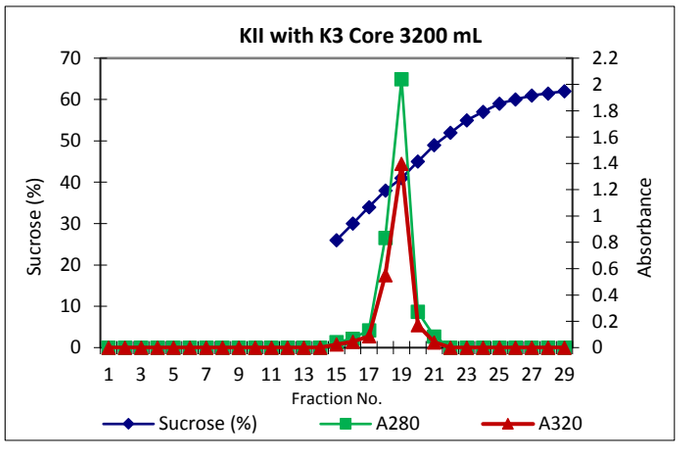
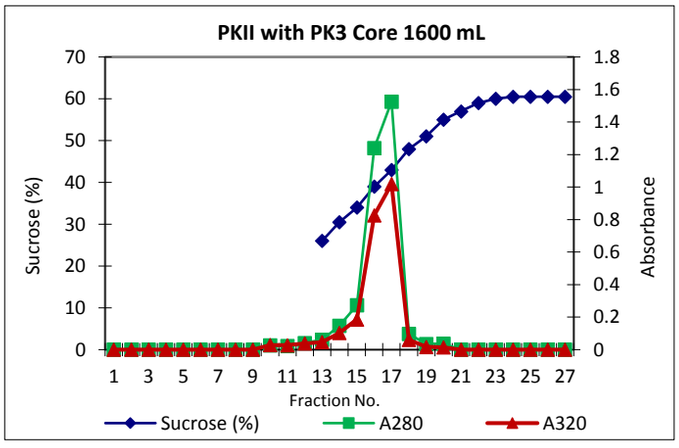
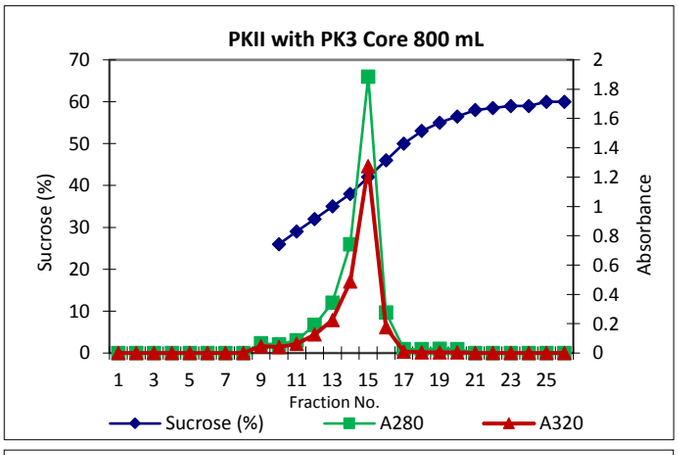
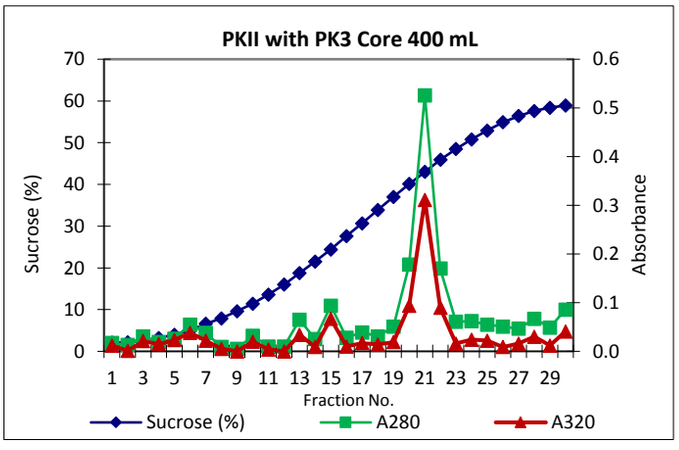
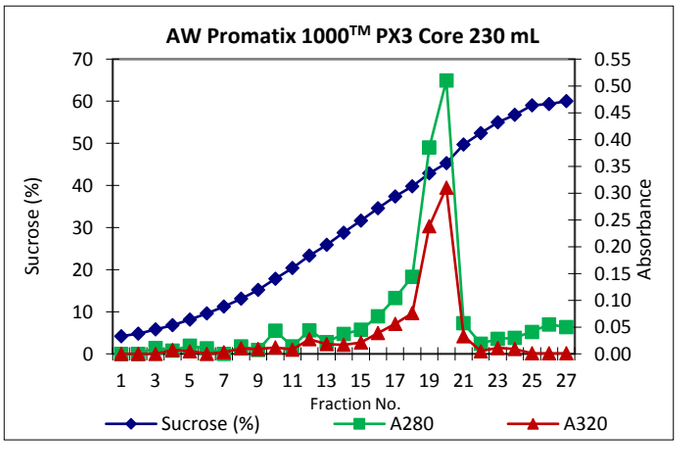
Linear Process Scaling











Separation parameters

Core	Core	Time To Sediment	Speed of Operation	K factor	Centrifugal Force Max	Centrifugal Force Min
Figure 6 at 3200 mL*	3200 mL	2.5 min	40500 rpm	29.7	121 200	100 000
Figure 17 at 1600 mL*	1600 mL	2.5 min	40500 rpm	29.7	121 200	100 000
Figure 18 at 800 mL*	800 mL	2.5 min	40500 rpm	29.7	121 200	100 000
Figure 19 at 400 mL*	400 mL	2.5 min	40500 rpm	29.7	121 200	100 000
AW Promatix 1000TM with 230 mL**	230 mL	3.36 min	35000 rpm	40	90 500	74 600

*See U.S. patent: 9,050,609. **See TAB: AW Promatix 1000TM 230mL Data

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Linear Process Scaling

Core	Core	Residence Time	Flow Through Volume	Flow Rate	Flow Rate
Figure 6 at 3200 mL*	3200 mL	3.4 min	1600 mL	28 L/h	466 mL/min
Figure 17 at 1600 mL*	1600 mL	3.4 min	800 mL	14 L/h	233 mL/min
Figure 18 at 800 mL*	800 mL	3.4 min	400 mL	7 L/h	116 mL/min
Figure 19 at 400 mL*	400 mL	3.4 min	200 mL	3.5 L/h	58 mL/min
AW Promatix 1000^{TM} with 230 mL**	230 mL	3.4 min	115 mL	1.5 L/h	25 mL/min

*See U.S. patent: 9,050,609. **See TAB: AW Promatix 1000TM 230mL Data

Peak analysis for each separation

Core	Core	Peak Recovery @ 25% threshold A_{280}	Peak Fraction (sucrose %)	Peak Density (g/cm^3)	Density Range @ 25% threshold (sucrose %)	Density Range (g/cm^3)
Figure 6 at 3200 mL*	3200 mL	83	41	1.1816	38 - 41	1.1663 - 1.1816
Figure 17 at 1600 mL*	1600 mL	79	43	1.1920	39 - 43	1.1713 - 1.1868
Figure 18 at 800 mL*	800 mL	70	42	1.1868	38 - 42	1.1663 - 1.1868
Figure 19 at 400 mL*	400 mL	72	43	1.2267	40.1 - 45.9	1.2113 - 1.2421
AW Promatix 1000^{TM} with 230 mL**	230 mL	76	45.3	1.2400	39.8 - 45.3	1.2100 - 1.2400

*See U.S. patent: 9,050,609. **See TAB: AW Promatix 1000TM 230mL Data

Core	Core	Polynomial Equation	R^2
Figure 6 at 3200 mL*	3200 mL	$y = -0.1636x^2 + 9.8708x - 86.211$	$R^2 = 0.9975$
Figure 17 at 1600 mL*	1600 mL	$y = -0.245x^2 + 12.342x - 94.675$	$R^2 = 0.9952$
Figure 18 at 800 mL*	800 mL	$y = -0.1541x^2 + 7.8075x - 38.879$	$R^2 = 0.9914$
Figure 19 at 400mL*	400 mL	$y = -0.0217x^2 + 3.0594x - 1.8911$	$R^2 = 0.9880$
AW Promatix 1000^{TM} with 230 mL**	230 mL	$y = 0.0343x^2 - 3.3571x + 68.701$	$R^2 = 0.9905$

*See U.S. patent: 9,050,609. **See TAB: AW Promatix 1000TM 230mL Data

Core	Core	Linear Equation	R^2
Figure 6 at 3200 mL*	3200 mL	$y = 3.7857x + 22.429$	$R^2 = 0.9989$
Figure 17 at 1600 mL*	1600 mL	$y = 4.25x + 21.786$	$R^2 = 0.9977$
Figure 18 at 800 mL*	800 mL	$y = 3.4048x + 21.929$	$R^2 = 0.9946$
Figure 19 at 400 mL*	400 mL	$y = 3.0412x + 18.533$	$R^2 = 0.9994$
AW Promatix 1000^{TM} with 230 mL**	230 mL	$y = 2.875x + 22.958$	$R^2 = 0.9971$

*See U.S. patent: 9,050,609. **See TAB: AW Promatix 1000TM 230mL Data

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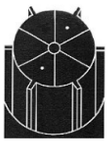
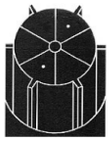
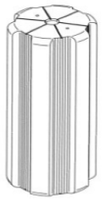
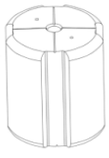
Rotor Assemblies for Density Gradient Centrifugation

Part Number	Rotor Type	Application	Max. Force	Capacity with Core	Dimensions
020330	 K3	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7	3.2 liters	Diameter: Max: 130mm Min: 110 mm Path Length: 11 mm
402509	 PK3	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7	1.6 liters	Diameter: Max: 130mm Min: 110 mm Path Length: 11 mm
402586	 PK3-800	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7	0.8 liters	Diameter: Max: 130mm Min: 110 mm Path Length: 11 mm
402575	 PK3-400	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7	0.4 liters	Diameter: Max: 130mm Min: 110 mm Path Length: 11 mm
403909 & 505061	 PX-230	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The basis of separation is the difference in buoyant densities of the particles being separated.	At 35 000 rpm Rmax: 90 500 xg Rmin: 74 600 xg K factor: 40	230 mL	Diameter: Max: 130 mm Min: 110 mm Path Length: 11 mm

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KII Ultracentrifuge Large-Scale Separation; 3200 mL scale

Regression fractions; 20-50% Sucrose	Fraction No.		Sucrose (%)	Sucrose (g/cm^3)	Absorbance (nm)	
					A_{320}	A_{280}
	30	1			0	0
	29	2			0	0
	28	3			0	0
	27	4			0	0
	26	5			0	0
	25	6			0	0
	24	7			0	0
	23	8			0	0
	22	9			0	0
	21	10			0	0
	20	11			0	0
	19	12			0	0
	18	13			0	0
	17	14			0	0
1	16	15	26.0	1.1082	0.025	0.042
2	15	16	30.0	1.1270	0.045	0.068
3	14	17	34.0	1.1463	0.087	0.132
4	13	18	38.0	1.1663	0.550	0.833
5	12	19	41.0	1.1816	1.397	2.041
6	11	20	45.0	1.2025	0.171	0.273
7	10	21	49.0	1.2241	0.040	0.085
	9	22	52.0	1.2406	0	0
	8	23	55.0	1.2575	0	0
	7	24	57.0	1.2690	0	0
	6	25	59.0	1.2806	0	0
	5	26	60.0	1.2865	0	0
	4	27	61.0	1.2924	0	0
	3	28	61.5	1.2954	0	0
	2	29	62.0	1.2983	0	0
	1	30	43.5	1.1973	0	0

25% of peak	0.5103
Peak recovery @ 25% threshold	83
Peak fraction Sucrose (%)	41
Peak density (g/cm^3)	1.1816
Density range @ 25% threshold Sucrose (%)	38 - 41
Density range @ 25% threshold Sucrose (g/cm^3)	1.1663 - 1.1816

Linear regression	**R^2 value**
$y = 3.7857x + 22.429$	$R^2 = 0.9989$

Polynomial analysis	**R^2 value**
$y = -0.1636x^2 + 9.8708x - 86.211$	$R^2 = 0.9975$

Rotor: AW P/N 020330

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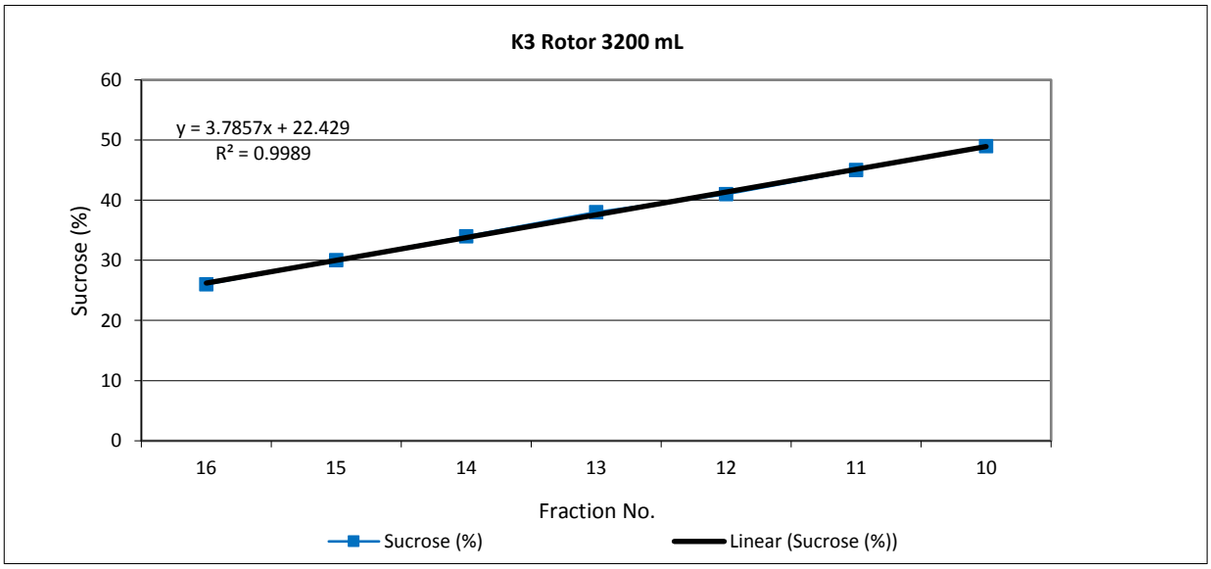
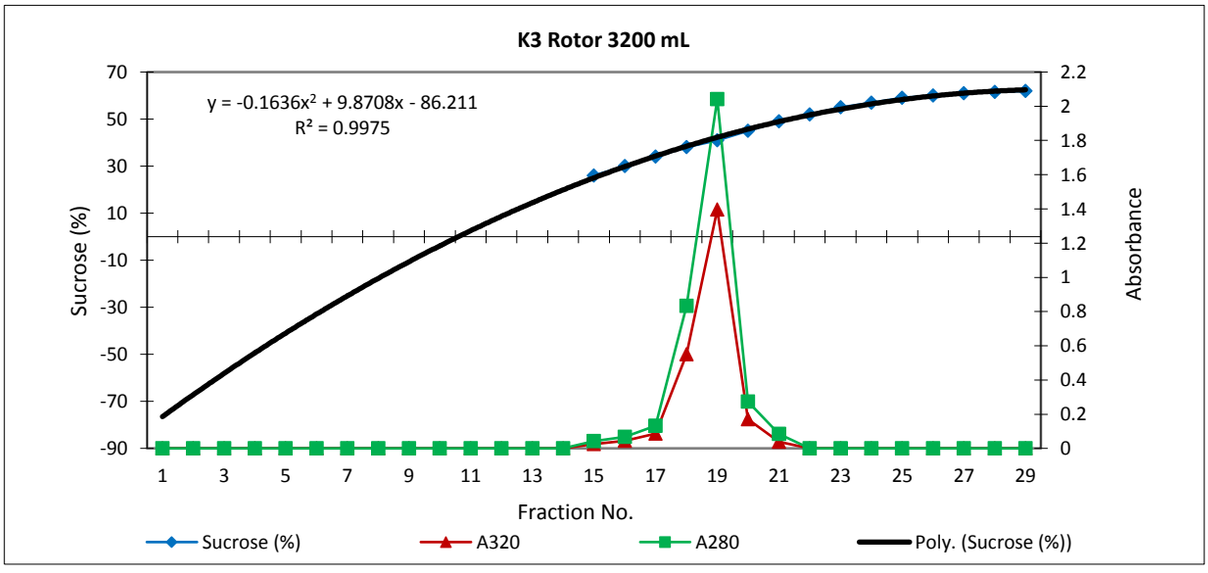
KII Ultracentrifuge Large-Scale Separation; 3200 mL scale

Method:

Merino, Sandra. 2015. Centrifuge with removable core for scalable centrifugation. U.S. Patent 9,050,609, filed November 30, 2010 and issued June 9, 2015.

NOTE: For the chart only data up to the maximum density is included.





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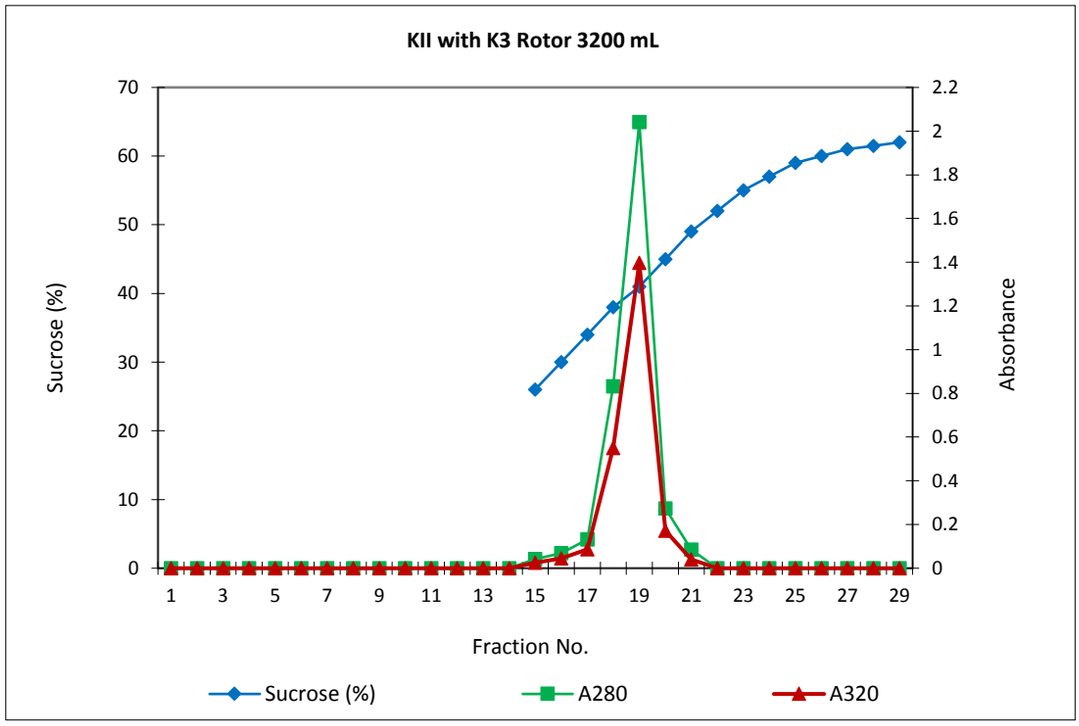
KII Ultracentrifuge Large-Scale Separation; 3200 mL scale



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PKII Ultracentrifuge Pilot-Scale Separation; 1600 mL scale

Regression fractions; 20-50% Sucrose	Fraction No.		Sucrose (%)	Sucrose (g/cm^3)	Absorbance (nm)	
					A_{320}	A_{280}
	30	2			0	0
	29	3			0	0
	28	4			0	0
	27	5			0	0
	26	6			0	0
	25	7			0	0
	24	8			0	0
	23	9			0	0
	22	10			0	0
	21	11			0.028	0.026
	20	12			0.027	0.023
	19	13			0.038	0.041
1	18	14	26.0	1.1082	0.046	0.060
2	17	15	30.5	1.1294	0.103	0.147
3	16	16	34.0	1.1463	0.186	0.274
4	15	17	39.0	1.1713	0.826	1.239
5	14	18	43.0	1.1920	1.019	1.526
6	13	19	48.0	1.2186	0.061	0.097
7	12	20	51.0	1.2351	0.017	0.035
	11	21	55.0	1.2575	0.014	0.036
	10	22	57.0	1.2690	0	0
	9	23	59.0	1.2806	0	0
	8	24	60.0	1.2865	0	0
	7	25	60.5	1.2895	0	0
	6	26	60.5	1.2895	0	0
	5	27	60.5	1.2895	0	0
	4	28	60.5	1.2895	0	0
	3	29	58.0	1.2748	0	0
	2	30	57.0	1.2693	0	0

25% of peak	0.3815
Peak recovery @ 25% threshold	79
Peak fraction Sucrose (%)	43
Peak density (g/cm^3)	1.1920
Density range @ 25% threshold Sucrose (%)	39 - 43
Density range @ 25% threshold Sucrose (g/cm^3)	1.1713 - 1.1920

Linear regression	**R^2 value**
$y = 4.25x + 21.786$	$R^2 = 0.9977$

Polynomial analysis	**R^2 value**
$y = -0.245x^2 + 12.342x - 94.675$	$R^2 = 0.9952$

Rotor: AW P/N 402509

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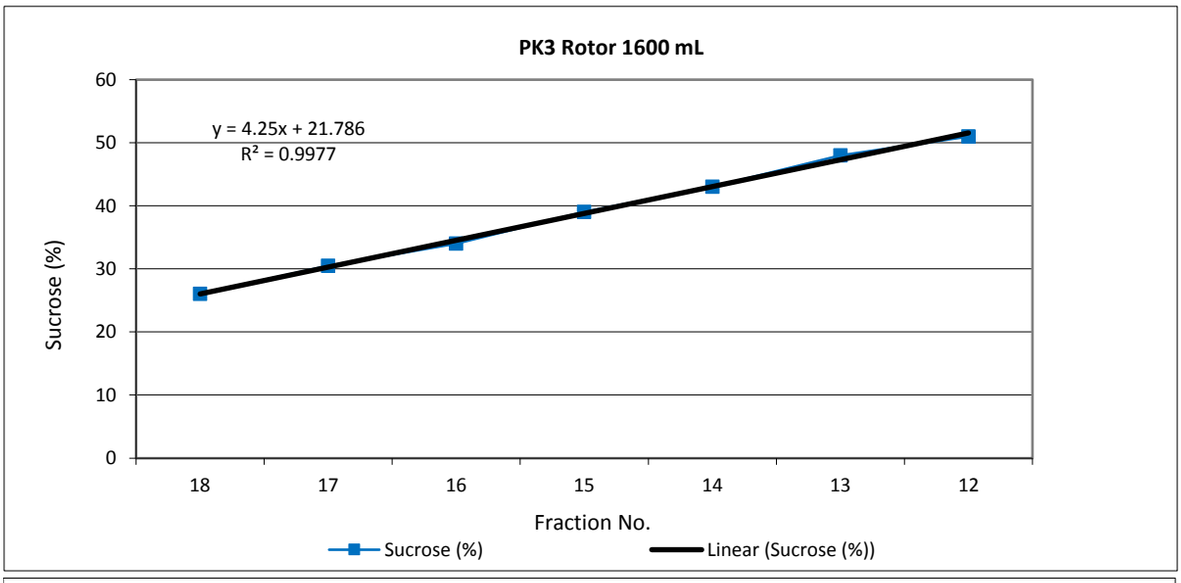
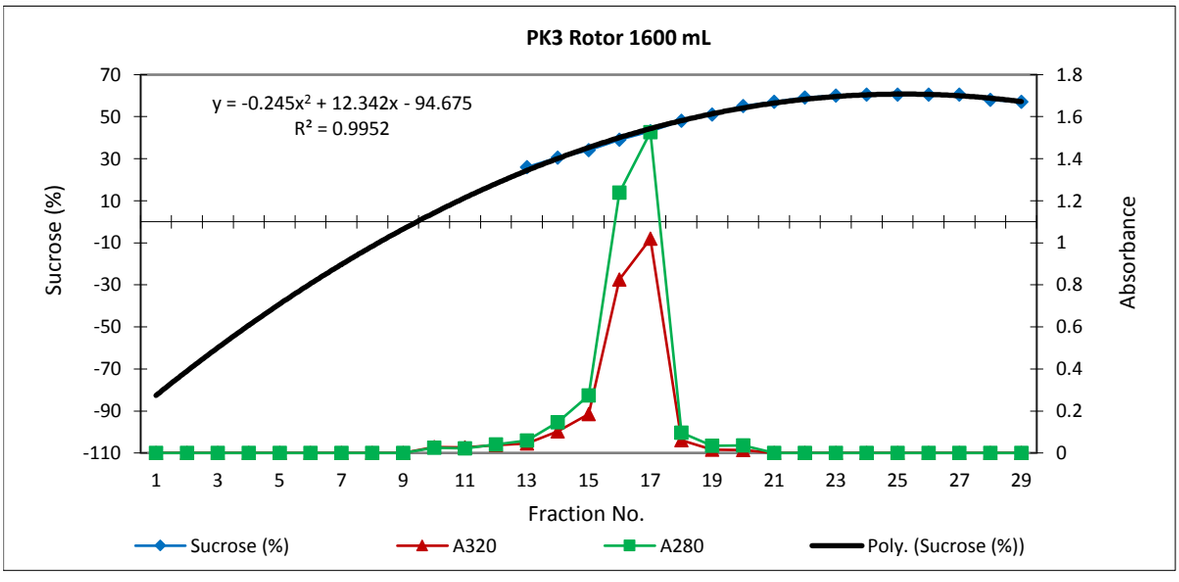
PKII Ultracentrifuge Pilot-Scale Separation; 1600 mL scale

Method:

Merino, Sandra. 2015. Centrifuge with removable core for scalable centrifugation. U.S. Patent 9,050,609, filed November 30, 2010 and issued June 9, 2015.

NOTE: For the chart only data up to the maximum density is included.





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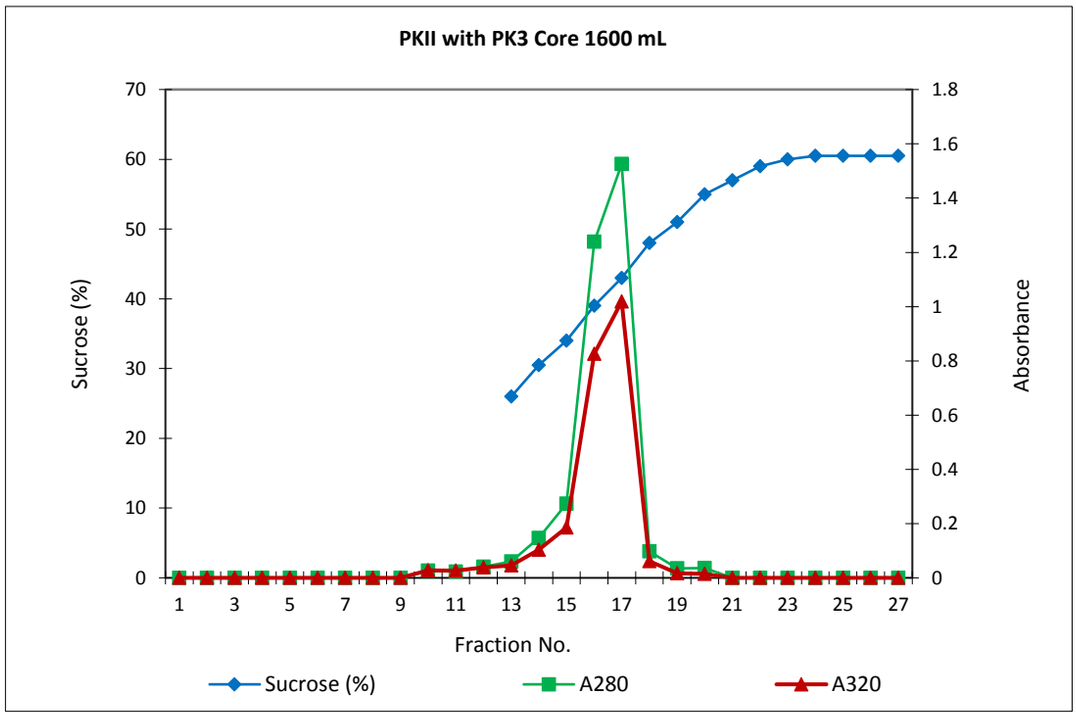
PKII Ultracentrifuge Pilot-Scale Separation; 1600 mL scale



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PKII Ultracentrifuge Pilot-Scale Separation; 800 mL scale

Regression fractions; 20-50% Sucrose	Fraction No.		Sucrose (%)	Sucrose (g/cm^3)	Absorbance (nm) A_{320}	A_{280}
	2	30			0	0
	3	29			0	0
	4	28			0	0
	5	27			0	0
	6	26			0	0
	7	25			0	0
	8	24			0	0
	9	23			0	0
	10	22			0.047	0.066
1	11	21	26.0	1.1082	0.040	0.059
2	12	20	29.0	1.1222	0.062	0.088
3	13	19	32.0	1.1366	0.126	0.191
4	14	18	35.0	1.1513	0.226	0.344
5	15	17	38.0	1.1663	0.487	0.742
6	16	16	42.0	1.1868	1.275	1.886
7	17	15	46.0	1.2079	0.176	0.277
8	18	14	50.0	1.2296	0.010	0.027
	19	13	53.0	1.2462	0.005	0.026
	20	12	55.0	1.2575	0.004	0.029
	21	11	56.5	1.2661	0.004	0.027
	22	10	58.0	1.2748	0	0
	23	9	58.5	1.2777	0	0
	24	8	59.0	1.2806	0	0
	25	7	59.0	1.2806	0	0
	26	6	60.0	1.2865	0	0
	27	5	60.0	1.2865	0	0
	28	4	59.5	1.2838	0	0
	29	3	58.5	1.2777	0	0
	30	2	42.0	1.1868	0	0

25% of peak	0.4715
Peak recovery @ 25% threshold	70
Peak fraction Sucrose (%)	42
Peak density (g/cm^3)	1.1868
Density range @ 25% threshold Sucrose (%)	38 - 42
Density range @ 25% threshold Sucrose (g/cm^3)	1.1663 - 1.1868

Linear regression	**R^2 value**
$y = 3.4048x + 21.929$	$R^2 = 0.9946$
Polynomial analysis	**R^2 value**
$y = -0.1541x^2 + 7.8075x - 38.879$	$R^2 = 0.9914$

Rotor: AW PN: 402586

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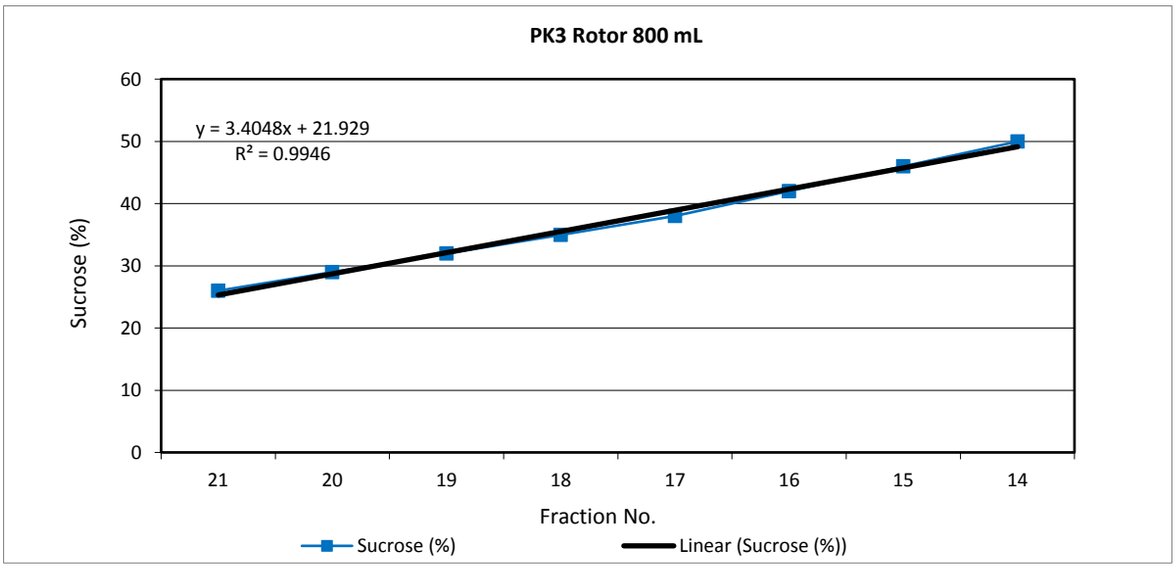
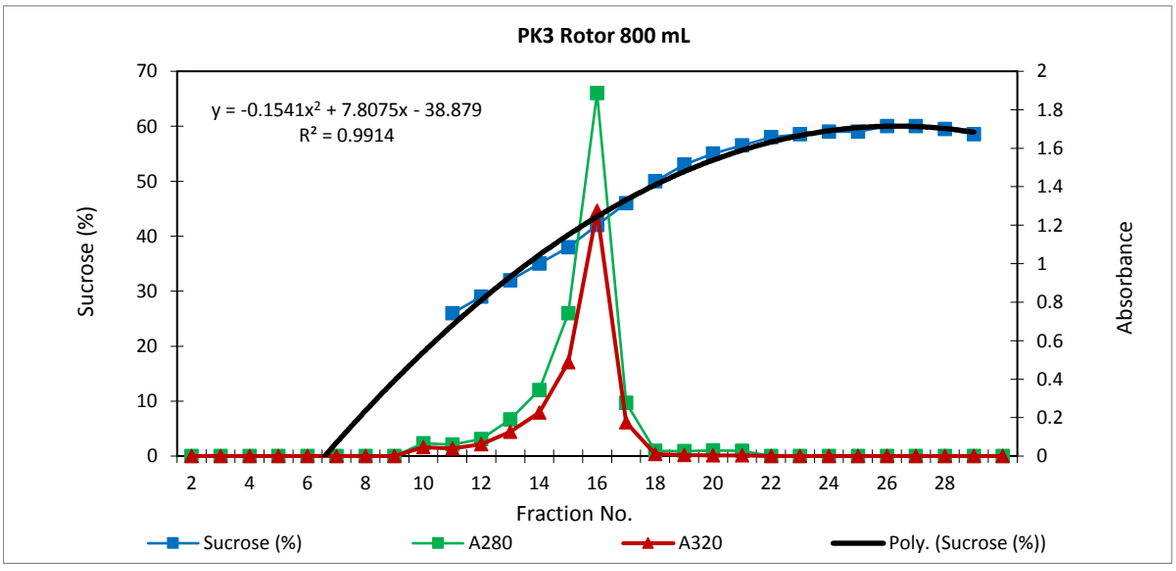
PKII Ultracentrifuge Pilot-Scale Separation; 800 mL scale

Method:

Merino, Sandra. 2015. Centrifuge with removable core for scalable centrifugation. U.S. Patent 9,050,609, filed November 30, 2010 and issued June 9, 2015.

NOTE: For the chart only data up to the maximum density is included.





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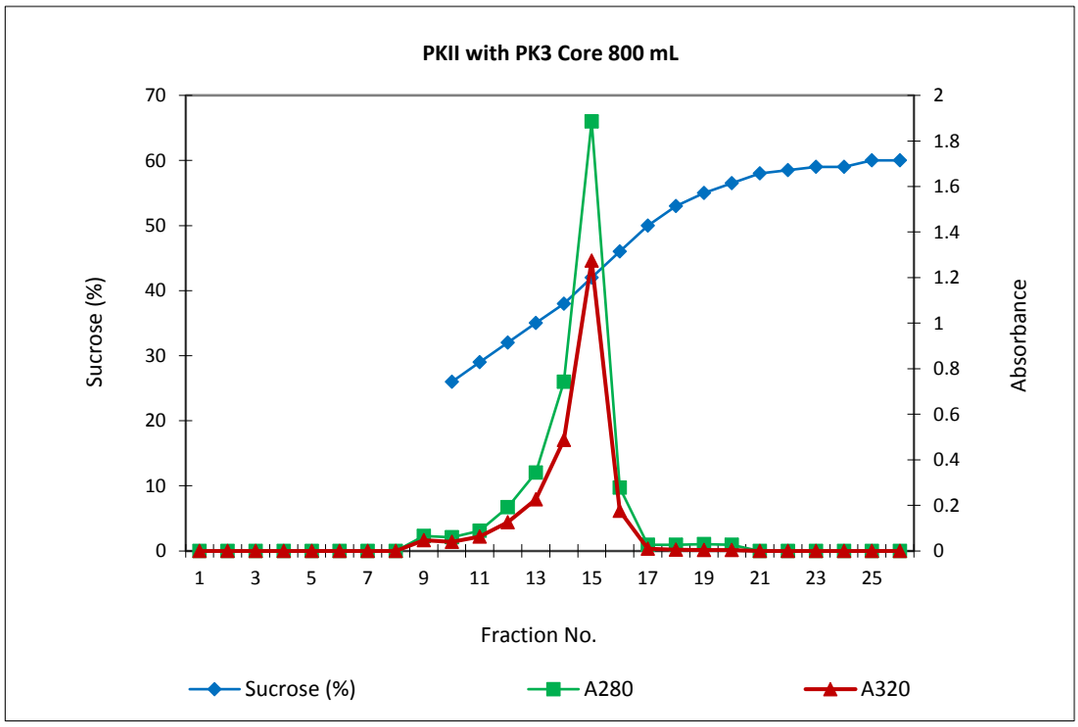
PKII Ultracentrifuge Pilot-Scale Separation; 800 mL scale



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PKII Ultracentrifuge Pilot-Scale Separation; 400 mL scale

Regression fractions; 20-50% Sucrose	Fraction No.		Sucrose (%)	Sucrose (g/cm^3)	Absorbance (nm)	
					A_{320}	A_{280}
	1	33	2.1	1.0099	0.012	0.017
	2	32	2.2	1.0105	0.001	0.013
	3	31	2.6	1.0126	0.021	0.031
	4	30	3.2	1.0158	0.015	0.019
	5	29	4.0	1.0200	0.023	0.026
	6	28	5.0	1.0253	0.038	0.055
	7	27	6.6	1.0338	0.022	0.038
	8	26	7.9	1.0407	0.005	0.009
	9	25	9.6	1.0497	0.000	0.005
	10	24	11.4	1.0592	0.020	0.032
	11	23	13.6	1.0709	0.004	0.010
	12	22	16.0	1.0836	0.000	0.010
	13	21	18.8	1.0984	0.034	0.065
1	14	20	21.5	1.1128	0.009	0.025
2	15	19	24.4	1.1281	0.067	0.094
3	16	18	27.6	1.1451	0.010	0.028
4	17	17	30.7	1.1615	0.017	0.039
5	18	16	33.9	1.1785	0.014	0.031
6	19	15	37.0	1.1949	0.019	0.051
7	20	14	40.1	1.2113	0.094	0.178
8	21	13	43.0	1.2267	0.311	0.526
9	22	12	45.9	1.2421	0.090	0.170
10	23	11	48.5	1.2559	0.016	0.061
	24	10	50.8	1.2680	0.024	0.062
	25	9	52.9	1.2792	0.022	0.055
	26	8	54.9	1.2898	0.009	0.051
	27	7	56.4	1.2977	0.016	0.047
	28	6	57.6	1.3041	0.030	0.067
	29	5	58.4	1.3083	0.012	0.049
	30	4	58.9	1.3110	0.041	0.086
	31	3	58.6	1.3094	0.024	0.062
	32	2	47.9	1.2527	0.001	0.030
	33	1	2.0	1.0094	0.018	0.024

25% of peak	0.1315
Peak recovery @ 25% threshold	73
Peak fraction Sucrose (%)	43
Peak density (g/cm^3)	1.2267
Density range @ 25% threshold Sucrose (%)	40.1 - 45.9
Density range @ 25% threshold Sucrose (g/cm^3)	1.2113 - 1.2421

Linear regression	**R^2 value**
$y = 3.0412x + 18.533$	$R^2 = 0.9994$

Polynomial analysis	**R^2 value**
$y = -0.0217x^2 + 3.0594x - 1.8911$	$R^2 = 0.9880$

Rotor: AW PN: 402575

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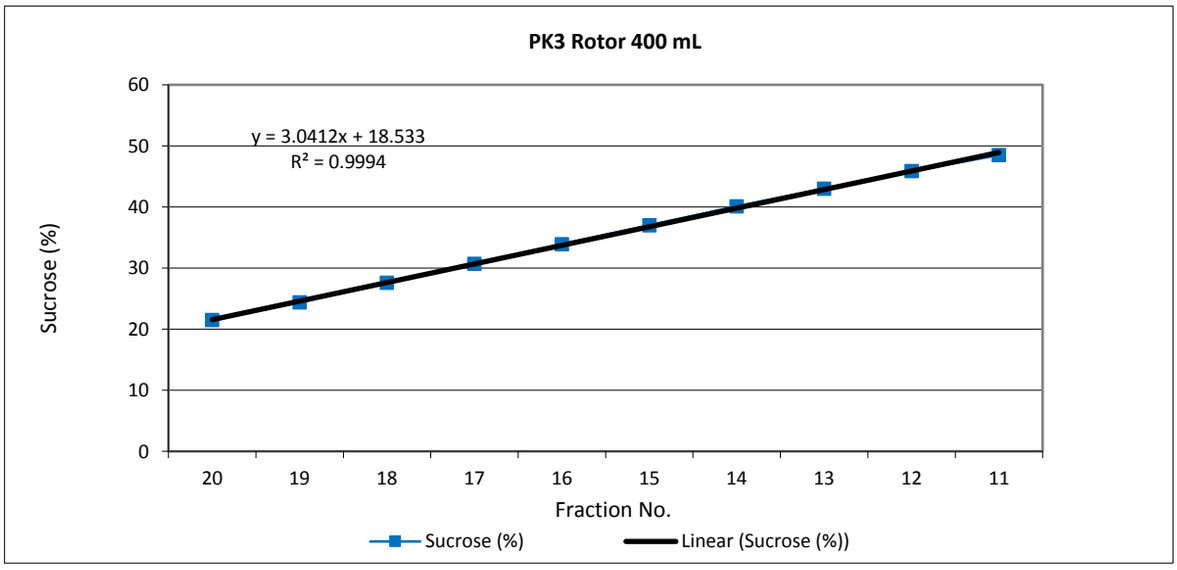
PKII Ultracentrifuge Pilot-Scale Separation; 400 mL scale

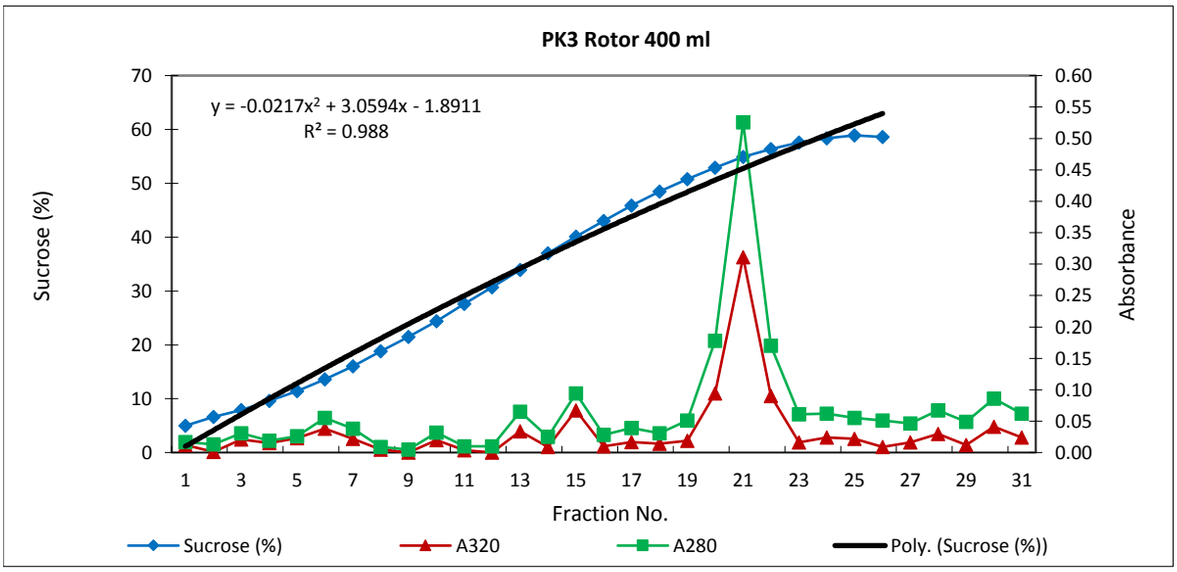
Method:

Merino, Sandra. 2015. Centrifuge with removable core for scalable centrifugation. U.S. Patent 9,050,609, filed November 30, 2010 and issued June 9, 2015.

Data Location: Lab Book P002 S Merino issue 12-10-03; page 71 03JUL2018

NOTE: For the chart only data up to the maximum density is included.





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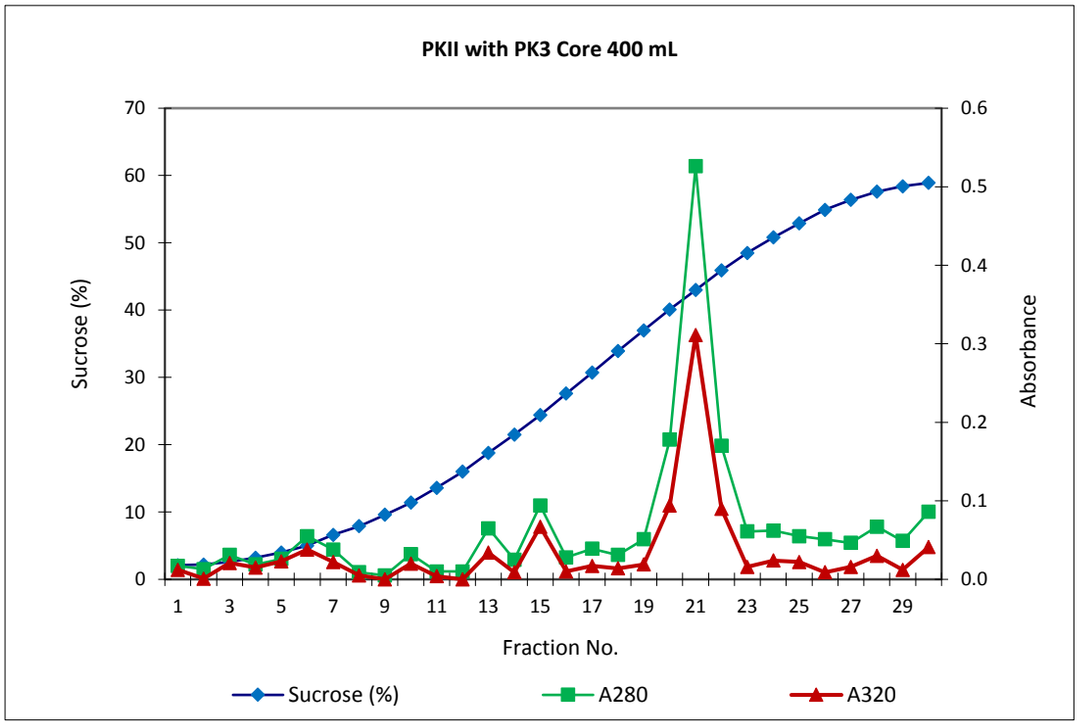
PKII Ultracentrifuge Pilot-Scale Separation; 400 mL scale



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AW Promatix 1000™ Ultracentrifuge Lab-Scale Separation; 230 mL scale

Regression fractions; 20-50% Sucrose	Fraction No.		Sucrose (%)	Sucrose (g/cm^3)	Absorbance (nm)	
					A_{320}	A_{280}
	1	32	3.2	1.0158	0.001	0.005
	2	31	3.6	1.0179	0.006	0.006
	3	30	4.2	1.0211	0.000	0.000
	4	29	4.8	1.0242	0.000	0.000
	5	28	5.8	1.0295	0.000	0.011
	6	27	6.8	1.0348	0.007	0.006
	7	26	8.1	1.0417	0.005	0.015
	8	25	9.6	1.0497	0.000	0.010
	9	24	11.2	1.0582	0.003	0.000
	10	23	13.1	1.0682	0.010	0.014
	11	22	15.2	1.0794	0.009	0.007
	12	21	17.8	1.0931	0.012	0.043
1	13	20	20.4	1.1069	0.008	0.014
2	14	19	23.3	1.1223	0.027	0.044
3	15	18	25.9	1.1361	0.018	0.022
5	16	17	28.8	1.1514	0.017	0.037
6	17	16	31.6	1.1663	0.021	0.045
7	18	15	34.6	1.1822	0.039	0.070
8	19	14	37.4	1.1970	0.056	0.104
9	20	13	39.8	1.2097	0.076	0.144
10	21	12	42.9	1.2262	0.238	0.385
11	22	11	45.3	1.2389	0.310	0.510
12	23	10	49.7	1.2622	0.033	0.057
	24	9	52.4	1.2765	0.005	0.019
	25	8	55.0	1.2903	0.011	0.028
	26	7	56.8	1.2998	0.009	0.030
	27	6	59.0	1.3115	0.001	0.041
	28	5	59.3	1.3131	0.001	0.055
	29	4	60.0	1.3168	0.001	0.050
	30	3	57.3	1.3025	0.003	0.054
	31	2	55.0	1.2903	0.003	0.062
	32	1	30.1	1.1583	0.009	0.033

25% of peak	0.1275
Peak recovery @ 25% threshold	76
Peak fraction Sucrose (%)	45.3
Peak density (g/cm^3)	1.2389
Density range @ 25% threshold Sucrose (%)	39.8 - 45.3
Density range @ 25% threshold Sucrose (g/cm^3)	1.2097 - 1.2389

Linear regression	**R^2 value**
$y = 2.875x + 22.958$	$R^2 = 0.9971$

Polynomial analysis	**R^2 value**
$y = 0.0343x^2 - 3.3571x + 68.701$	$R^2 = 0.9905$

Rotor: AW PN: 403909 + 505061

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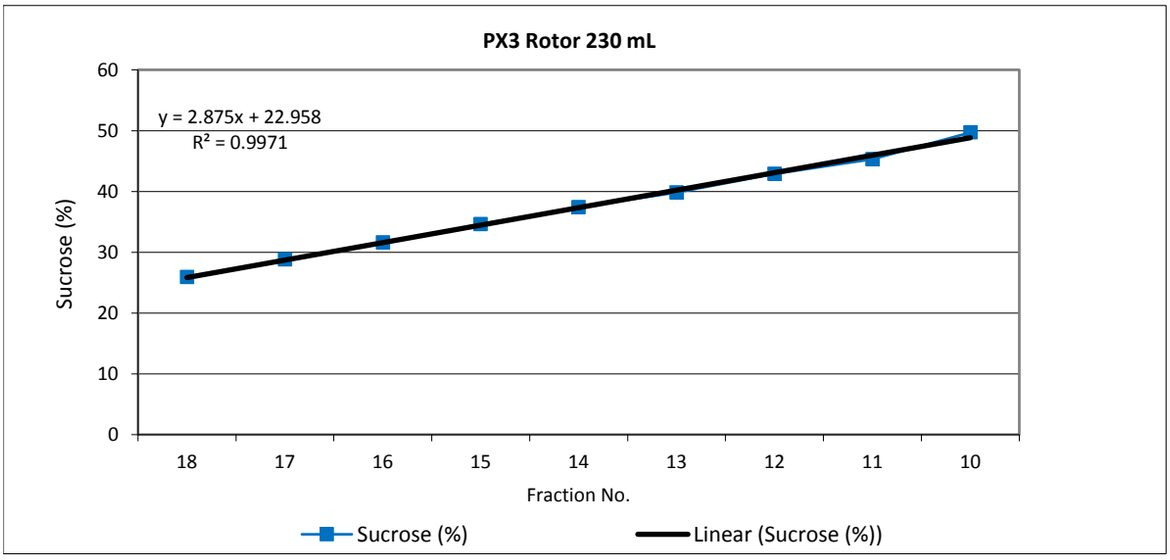
AW Promatix 1000™ Ultracentrifuge Lab-Scale Separation; 230 mL scale

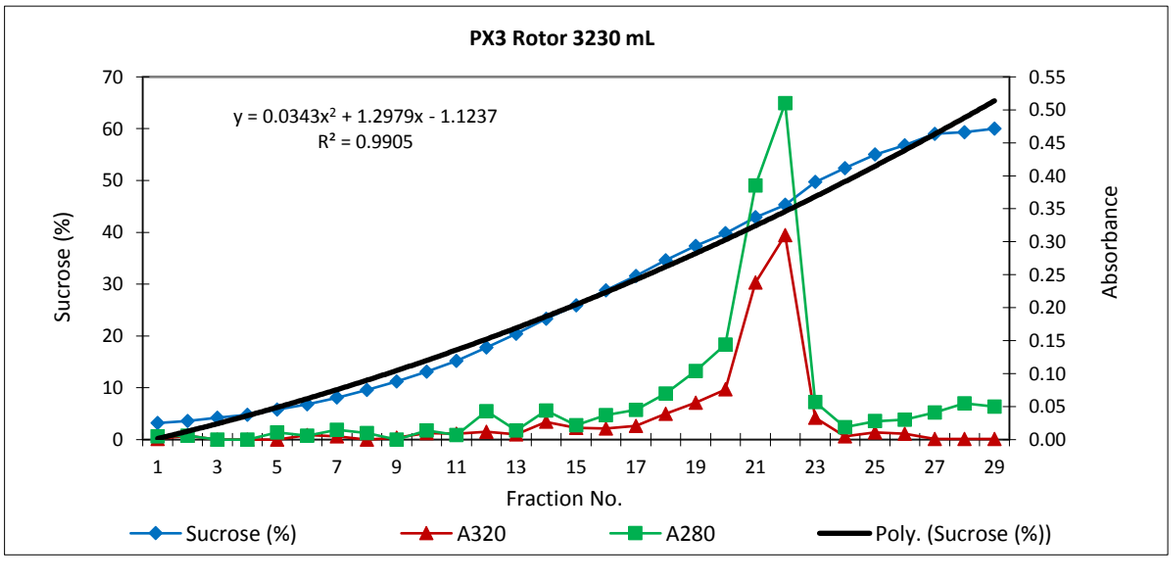
Method:

Merino, Sandra. 2015. Centrifuge with removable core for scalable centrifugation. U.S. Patent 9,050,609, filed November 30, 2010 and issued June 9, 2015.

Data Location: Lab Book P002 S Merino issue 12-10-03; page 71 03JUL2018

NOTE: For the chart only data up to the maximum density is included.





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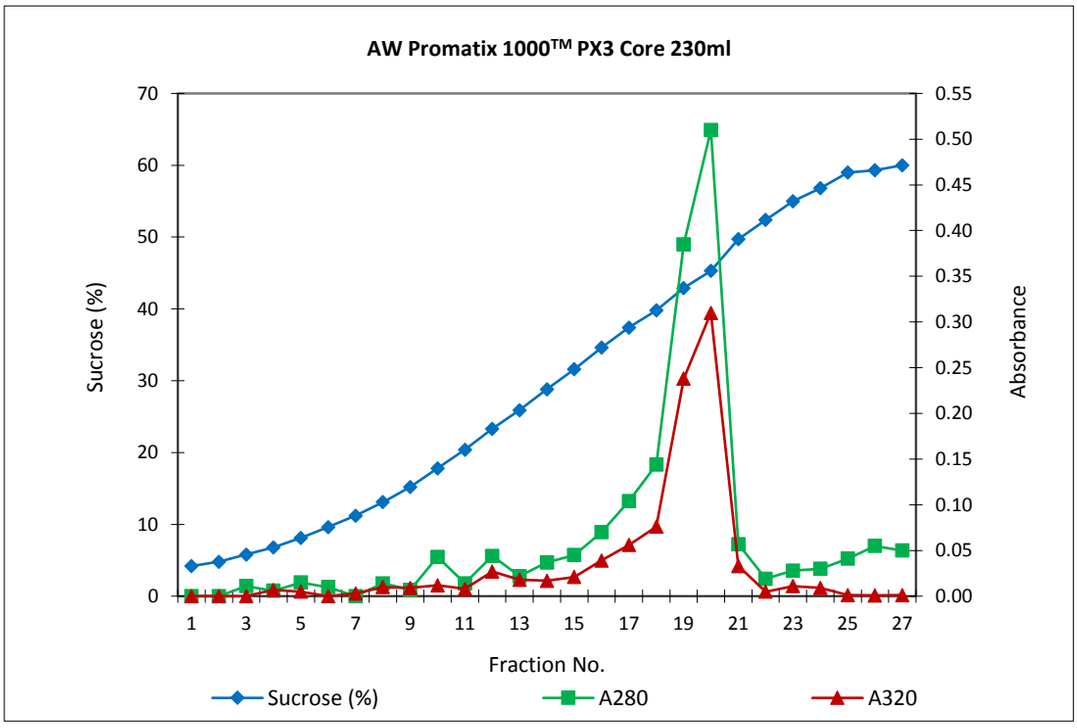
AW Promatix 1000™ Ultracentrifuge Lab-Scale Separation; 230 mL scale



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