Octave™ PQ Kit

Cat. No. A1017K

For more detailed information please refer to the Semba Biosciences website www.sembabio.com.

DESCRIPTION

The Octave™ PQ Kit is used to test separation of two compounds by simulated moving bed chromatography (SMBC) on Semba's Octave System. Size exclusion is used to separate Blue Dextran (M_r ~2 million) from cyanocobalamin (Vitamin B12; M_r 1,355), where the feed mixture (purple in color) separates into blue and red raffinate and extract streams, respectively. The supplied 1-ml columns contain Toyopearl® HW 65F, 30 - 60 micron particles. A 3-2-3 Isocratic mode SMBC script is used.

KIT COMPONENTS

C1005S Octave PQ Column, Set of 8

R1026E Octave PQ Feed Solution, 100 ml

R1022E Octave PQ Desorbent Solution, 10X, 100 ml

3-2-3 ISOCRATIC MODE

Refer to the Octave User Manual for a discussion of SMBC principles and operation of the instrument. In the 3-2-3 configuration Zone 1 consists of three columns for recovering slow moving component A and cleaning the solid matrix; Zone 2 contains two columns for concentration of A and exclusion of B, and Zone 3 contains three columns for inclusion of A and concentration of B. All raffinate exits the system after Zone 3 due to the closed shutoff valve between Zones 3 and 1.

For this separation the purple feed mixture resolves into red and blue components for a simple visual demonstration of SMBC. The small molecule cyanocobalamin is slowed by inclusion within the resin particles and exits as a pink to red solution from the extract port. The large molecule Blue Dextran is excluded from the pores of the resin and flows rapidly through the columns exiting as a blue solution through the raffinate port.

PARAMETERS

Pre-programmed scripts optimized for this separation (PQ Script 1, PQ Script 2) are included with the SembaPro™ software application. Flow rates and switch times for both scripts are listed in the table below. The scripts vary only in the extract flow rate; the slower rate in PQ Script 1 produces higher purity extract.

	PQ Script 1	PQ Script 2
Pump 1 (Feed)	0.4 ml/min	0.4 ml/min
Pump 2 (Desorbent)	2.0 ml/min	2.0 ml/min
Pump 3 (idle)	0	0
Pump 4 (Extract)	0.90 ml/min	1.1 ml/min
Switch Time	48 sec	48 sec

RUNNING THE PQ SCRIPTS

Refer to the Octave User Manual for this step. Set up pump connections for isocratic mode SMBC, where Pumps 1 and 2 are connected to inlets A and B, respectively, Pump 3 is not connected, and outlet E (extract) is connected to the inlet of Pump 4.

1. Connect tubing to outlet F (raffinate) and to the outlet of Pump 4 (extract). Insert plugs into outlets G and H. Install a 100 psi backpressure regulator on the line after the Pump 4 outlet and a 40 psi backpressure regulator on the raffinate outlet line. Set up appropriate collection vessels.
2. If the system has not been purged previously, connect jumpers as described in Section 3. Run PQ Script 1 to purge the system.
3. Connect columns as described in the User Manual.
4. Prepare sufficient amounts of 1X Desorbent Solution (dilute 10X stock with deionized water) and Feed Solution, and filter solutions through 0.45 micron filters.
5. Place Pump 1 (Feed) and Pump 2 (Desorbent) inlet lines into the Desorbent Solution reservoir. Prime the pumps with a syringe as described in the User Manual.
6. Run PQ Script 1 with 1X Desorbent Solution instead of Feed for one cycle to equilibrate the system.
7. Stop the script, place the Feed inlet into the Feed solution, and use a syringe to draw the Feed into Pump 1.
8. Run PQ Script 1 for 5 cycles (run time = 32 minutes). Collect extract and raffinate into separate vessels. After 4 cycles switch to fresh vessels and collect the outlet streams from the fifth cycle.
9. Stop the script, then run PQ Script 2 for 5 cycles, collecting streams as in Step 8.
10. The colors of the extract and raffinate collected from the fifth cycle should be red/purple respectively, for PQ Script 1 and purple/blue respectively, for PQ Script 2. Product streams can be analyzed at 535 nm for cyanocobalamin and 620 nm for Blue Dextran. A 1:5 water dilution of the purple PQ standard will have an A_{620}/A_{535} ratio of approximately 0.915. The blue raffinate A_{620}/A_{535} ratio should be ≥2.30 and the red extract A_{620}/A_{535} ratio should be ≤0.40.
11. After the run, rinse the system with water for at least 3 cycles and then with 20% ethanol for another 3 cycles. Remove the columns, install jumpers, purge the system once more and shut down the system.

STORAGE

Store kit components at 4°C. Do not allow the columns to freeze!

www.sembabio.com Toll-free 866.634.1114 Fax 608.441.8329