
8. SMBC PROTOCOLS FOR PROTEIN PURIFICATION

This section provides abbreviated protocols and a listing of conditions and scripts included with the Octave System for common protein purification procedures. See Section 7 for detailed information on the step and Isocratic SMBC modes of operation.

See Section 12 (Appendix 3) for protocols for preparation of protein samples from bacterial cell cultures and estimation of target protein concentration.

8.1. Step SMBC for affinity purification

The following protocol can be applied to a variety of purification chemistries, such as IMAC of histidine-tagged proteins, glutathione affinity chromatography of GST-fusion proteins, and Protein A/Protein G affinity chromatography of antibodies.

1. Set up pump connections as shown in Section 2.7.2, Figure 3, where Pumps 1-4 are connected to inlets A-D, respectively.
2. Connect tubing to outlets E-H. Outlet E will be the purified product, so it can be run through an appropriate detector. All outlets should have a minimum 40 psi backpressure (maximum 270 psi); when running 1-ml or 5-ml polymeric columns (particle diameter > 30 microns), use the 40 psi backpressure regulators supplied with the system. Set up appropriate collection vessels. Unbound material (raffinate) will come from outlet F, G will contain wash effluent, and H will contain equilibration buffer used following elution.
3. Purge the system using jumpers as described in Section 3.
4. Connect columns as described in Section 6.
5. Prepare sufficient amounts of all solutions: Feed, Wash, Elute, Regenerate (Equilibrate). Filter all solutions through 0.45 micron filters before running through the Octave System.

For IMAC using Ni-NTA Superflow columns, use these formulations:

Feed (sample) and Regenerate: 50 mM Na phosphate, pH 8.0, 300 mM NaCl, 10 mM imidazole

Wash: 50 mM Na phosphate, pH 8.0, 300 mM NaCl, 20 mM imidazole

Elute: 50 mM Na phosphate, 300 mM NaCl, 250 mM imidazole

6. Estimate the concentration of target protein in the cell lysate or other sample to be used as Feed solution.
7. Select a script from the chart below based on the target protein concentration and columns being used. See Appendix 3 for information on estimating target protein expression levels.

1-ml columns, 20 mg/ml capacity

Script Name	Step-1ml-20-0.25	Step-1ml-20-0.5	Step-1ml-20-1	Step-1ml-20-2	Step-1ml-20-3
Expression level (mg/ml target in Feed)	0.25	0.5	1.0	2.0	3.0
Target protein concentration (mg/ml)	0.3	0.75	1.5	3.0	4.5
Feed flow rate (ml/min)	3.0	3.0	2.0	1.5	1.0
Wash flow rate (ml/min)	0.6	1.5	2.0	3.0	3.0
Elute flow rate (ml/min)	0.18	0.45	0.6	0.9	0.9
Regenerate flow rate (ml/min)	0.6	1.5	2.0	3.0	3.0
Switch time (sec)	1000	400	300	200	200
Theoretical yield (mg/h)	54	135	180	270	270
Target protein conc in eluate (mg/ml)	5.0	5.0	5.0	5.0	5.0

1-ml columns, 5 mg/ml capacity

Script Name	Step-1ml-5-0.25	Step-1ml-5-0.5	Step-1ml-5-1	Step-1ml-5-2	Step-1ml-5-3
Expression level (mg/ml target in Feed)	0.25	0.5	1.0	2.0	3.0
Target protein concentration (mg/ml)	0.3	0.75	1.5	3.0	4.5
Feed flow rate (ml/min)	2.0	1.6	1.2	1.0	0.67
Wash flow rate (ml/min)	1.6	3.2	4.8	8.0	8.0
Elute flow rate (ml/min)	0.48	0.96	1.44	2.4	2.4
Regenerate flow rate (ml/min)	1.6	3.2	4.8	8.0	8.0
Switch time (sec)	375	187.5	125	75	74.6
Theoretical yield (mg/h)	36	72	108	180	180.9
Target protein conc in eluate	1.25	1.25	1.25	1.25	1.25

5-ml columns, 20 mg/ml capacity

Script Name	Step-5ml-20-0.25	Step-5ml-20-0.5	Step-5ml-20-1	Step-5ml-20-2	Step-5ml-20-3
Expression level (mg/ml target in Feed)	0.25	0.5	1.0	2.0	3.0
Target protein concentration (mg/ml)	0.3	0.75	1.5	3.0	4.5
Feed flow rate (ml/min)	10.0	8.0	5.0	4.0	2.7
Wash flow rate (ml/min)	2.0	4.0	5.0	8.0	8.1
Elute flow rate (ml/min)	0.60	1.2	1.5	2.4	2.43
Regenerate flow rate (ml/min)	2.0	4.0	5.0	8.0	8.1
Switch time (sec)	1500	750	600	375	370
Theoretical yield (mg/h)	180	360	450	720	729
Target protein conc in eluate (mg/ml)	5.0	5.0	5.0	5.0	5.0

8. Connect all inlet lines to appropriate reservoirs except use Bind buffer for Pump 1 (Feed). Prime the pumps with a syringe as described in Section 3.5.
9. Run the appropriate script with Bind buffer instead of Feed for one cycle to equilibrate the system.
10. Transfer the Pump 1 inlet to the Feed reservoir and run the script for the desired length of time.
11. During the second cycle, analyze samples of Extract, Raffinate, Wash effluent, and (if desired) Regeneration effluent for the target protein.
12. Adjust running parameters (flow rates, switch time) based on results. Guidelines for adjustments are given in the table below.

Symptom	Possible reason	Possible solutions/steps
Target protein in Raffinate (flow-through); no other problem	Inefficient binding or target protein concentration in Feed too high for script conditions	Decrease Feed flow rate; ensure column is properly equilibrated in Bind buffer; ensure target protein concentration is in appropriate range; switch to another script for lower target protein concentration
Target protein in Raffinate (flow-through); poor yield in Extract	Loss of column binding capacity	Ensure columns are fully charged (IMAC); ensure correct buffer formulations; ensure that columns are fully equilibrated in Bind buffer in Regeneration zone (increase flow rate of Pump 4); test an individual column for ability to bind target
Insufficient purity of target protein	Stringency/duration of Wash zone too low	Increase flow rate of Pump 2 (Wash); increase [imidazole] in Wash (IMAC); increase switch time
Target protein in Regeneration effluent	Incomplete elution in Elute zone	Increase flow rate of Pump 3 (Elute)

8.2. Isocratic SMBC for affinity purification

When used for IMAC protocols, isocratic mode gives superior purity with the convenience of a single running buffer. Since there is usually a several-fold dilution using this method, it is not recommended for purification of proteins at starting concentrations less than 0.5 mg/ml. The script 3-2-3 Iso-1ml can be run for many different applications and columns, independent of target protein concentration (above the recommended 0.5 mg/ml). Samples can be concentrated either before or after the isocratic run using a Step mode protocol as in the previous section (not that appropriate adjustment of the imidazole concentration is needed for this to apply in IMAC applications).

1. Set up pump connections as shown in Section 7.6, Figure 15, 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.
2. Connect tubing to outlet F (Raffinate; unretained protein) and to the outlet of Pump 4 (Extract; target protein). Insert plugs into outlets G and H. Raffinate and Extract lines can be run through detectors. The Extract line is metered by Pump 4 and must be under the highest back pressure in the system to effectively control the flow rate. Most often a 100 psi backpressure regulator (supplied with the system) is inserted downstream of the detector. Backpressure downstream of Pump 4 must exceed maximum system pressure by at least 35 psi. Set up appropriate collection vessels.
3. If the system has not been purged previously, connect jumpers as described in Section 3. Run the 3-2-3 Iso-1ml script to purge the system.
4. Connect columns as described in Section 6.
5. Prepare sufficient amounts of running buffer and Feed, making sure to bring the Feed to the same composition as the running buffer. Filter solutions through 0.45 micron filters before running through the Octave System.

For IMAC using Ni-NTA Superflow columns for purification of 6xHis-fusion proteins, use this formulation for both the Feed and Desorbent: 50 mM Na phosphate, pH 8.0, 300 mM NaCl, 75 mM imidazole

6. Place Pump 1 (Feed) and Pump 2 (Desorbent) inlet lines in the running buffer reservoir. Prime the pumps with a syringe as described in Section 3.5.
7. Run the 3-2-3 Iso-1ml script with running buffer instead of Feed for one cycle to equilibrate the system. See the table below for script conditions and theoretical yields for various concentrations of target protein in the Feed. Note that these are theoretical yields only; actual yields will vary based on the sample, columns, and running conditions.

Script: 3-2-3 Iso-1ml

Expression level (mg/ml target in Feed)	0.5	1.0	2.0	3.0
Target protein concentration (mg/ml)	0.75	1.5	3.0	4.5
Feed flow rate (ml/min)	0.8	0.8	0.8	0.8
Desorbent flow rate (ml/min)	2.0	2.0	2.0	2.0
Extract flow rate (ml/min)	1.1	1.1	1.1	1.1
Raffinate flow rate (ml/min)	1.7	1.7	1.7	1.7
Switch time (sec)	105	105	105	105
Theoretical yield (mg/h)	36	72	144	216
Target protein conc in eluate (mg/ml)	0.54	1.09	2.18	3.27

8. Transfer the Pump 1 inlet to the Feed reservoir and run the script for the desired length of time.
9. Analyze Extract and Raffinate samples for target protein, and make adjustments to the script as required (see Sections 7.6.2, 7.6.5).