Gradating Dye Chromatography (Kit No. RBA-4 & RR120-4)

Reactive dye chromatography is a versatile tool in protein purification. Sigma offers pre-packed columns with degrees of dye substitution varying from 0.1 μM to 3 μM or more per ml gel to determine the optimum dye-resin substitution when designing a reliable and effective purification protocol.

Because of the non-specific nature of protein-dye interactions, the formulation of a purification scheme should be designed so that several parameters are kept as constant as possible. These parameters include:

1) Buffer systems
   A) The pH and ionic strength of the equilibrated column and the protein solution should match as nearly as possible.
   B) The buffer-protein compatibility (buffers should be non-denaturing to protein function).
   C) The resin-buffer compatibility [buffers should be specific for the desired function of the resin (i.e., high ionic strength for hydrophobic interaction or low ionic strength for affinity interaction)].

2) Protein concentration
   A) The load solutions should be a constant mg protein/ml resin (This point is particularly critical). In cases of very low protein concentrations and very low mg protein/ml resin ratios, irreversible binding can occur.
   B) In some applications, the consistent loading of units/ml resin may be more reproducible.
   C) Differences in specific activity of load solutions can substantially alter the resulting chromatography.

Procedure: [Note: Temperature optimum may vary/Recommended temperature 3-8°C]

1) Equilibrate the column or columns with 5-10 column volumes of the chosen buffer.
2) Load the protein solution (concentration should be 1-10 mg/ml) to the column. (In comparing columns it is generally advisable to load equal quantities of protein to each column).
3) Wash the load into the column with 0.1 ml to 0.5 ml equilibration buffer.
4) Continue to wash the column with equilibration buffer (5-10 column volumes) until no protein leakage is observed.

5) Elute the target protein with the chosen eluant. Suggested eluting conditions:

A) ionic strength (increasing salt concentrations) (0.05 M to 2.0 M)

B) competitive elution (coenzymes, cofactors, substrates) (5 mM to 50 mM)

C) pH shifts

D) chaotropic agents (urea, guanidine, Sodium thiocyanate, etc.) (0.5 M to 6 M)

6) Evaluate binding capacity vs total recovery to determine:

A) maximum binding effectiveness for differing levels of dye substitutions

B) maximum recovery

C) ease of recovery

D) degree of purification

Example: A theoretical composite based on a variety of results from a diverse sample of proteins. Data actually obtained varies from protein to protein. The most effective resin can be determined by individual results. In this example resin 1000 is the most efficient resin based on % recovery and degree of purity.

	Resin 3000	Resin 1000	Resin 300	Resin 100
Column Size	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Load 3 ml at 10 mg/ml 10 unit/ml	specific activity = 1 unit/mg			
units bound	30	25	20	15
units recovered	24	24	15	4.5
mg recovered	12	6	4.2	1.5
Purification factor	2X	4X	3.5X	3X

7) Regenerate the column as directed below.

Regeneration:

Wash the column with 10 column volumes of each:

1) 0.1M Borate pH 9.8 + 1.0 M NaCl
2) 0.1 M Borate pH 9.8
3) Deionized water or distilled water.
4) 2.0 M NaCl

Storage:

Store column upright with both caps in place at 3-8°C. 0.01 to 0.02% Thimerosal may be added for long term storage. **DO NOT FREEZE!**

Sigma offers individual dye-agarose columns for further testing and larger package sizes for scale-up. Bulk quantities of dye-resins are also available upon inquiry.