**Title: Column Chromatography of Green Fluorescent Protein**

Approvals:

Preparer Date\_07Oct06\_\_\_\_\_\_\_\_Reviewer: Mary Jane Kurtz \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Date\_\_\_\_\_\_\_\_\_\_

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**Part I**

**Crude Isolation of GFP from Lysed Cells**

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**1. Purpose:**

1.1 The purpose of this SOP is to purify green fluorescent protein (GFP) from lysed *E*. *coli* cells using column chromatography.

**2. Scope:**

 2.1 This SOP will be executed whenever the purification of GFP is needed.

* 1. This SOP will be executed after the performance of pGLO Bacterial Transformation.

**3. Responsibility:**

3.1 It is the responsibility of the Instructor to aid the students in the performance of all the procedures described in this SOP.

 3.2 It is the responsibility of the Instructor to update this SOP as needed.

**4. References:**

4.1 Instructions from Biotechnology Explorer Green Fluorescent Protein (GFP) Purification Kit Instruction Manual (Bio-Rad catalog number 166-0005EDU

 4.2 GFP Chromatography Kit. (Bio-Rad catalog number 166-0005EDU)

**5. Definitions:**

 5.1 Ion Exchange Chromatography

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5.2 Hydrophobic Interaction Chromatography

 5.4 Elution

 5.5 Eluate

5.6 Column Volume5.7 Supernatant

**6. Hazard Communication**

 6.1 Wear personal protection equipment

 6.2 Wear eye protection.

6.3 For Disposal of Biohazard Waste use an autoclavable bag and follow proper sterilization techniques.

**7. Materials**

 7.1 Safety glasses or goggles

 7.2 Microfuge tubes

 7.3 Pipettes

 7.4 Microtube rack

 7.5 Marker

 7.6 *E. coli* GFP cell culture from Bacterial Transformation and Upstream Processing
 SOP
7.7 Incubator to grow *E. coli* transformed with GFP at 35C
7.8 Freezer
7.9 Microfuge centrifuge
7.10 Vortex7.11 TE Buffer
7.12 Lysozyme in 1ml of TE Buffer
7.13 UV Lamp

**8. Procedure:**

* 1. **Preliminary Procedures before Chromatography**
		1. Take 2ml of pGLO transformed *E.coli* culture from the 20ml culture tube, centrifuge at 2,000 RPM for twenty minutes. The cell pellet should fluoresce when viewed by a UV lamp.



* + 1. While waiting, rehydrolyze the lysozyme with 1 ml of TE buffer.
		2. Pour off the *E. coli* supernatant into a 10% Clorox solution.

8.1.5 Add 750 l of TE buffer and resuspend the pellet by pipetting the fluid up

 and down many times to make a homogenous mixture. Vortex if

 necessary.

8.1.6 Add 1 drops of the reconstituted lysozyme solution. Mix the contents

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 gently. Freeze contents at -20C for 1 day at least.

8.1.7 Thaw the microfuge tube containing bacterial cell lysate to hand warmth
 temperature.

8.1.8 Centrifuge for 10 minutes at maximum speed.

8.1.9 Observe the bacterial cell supernatant with the UV light. It should
 fluoresce.

8.1.10 Remove the bacterial cell lysate supernatant and place into a clean
 microfuge tube. This is the fraction we will use to purify GFP.

**Part II**

 **Hydrophobic Interaction Chromatography**

**9.0 Materials**

 9.1 250ul of bacterial cell lysate

9.2 Pre-packed Hydrophobic Interaction Chromatography Column

 9.3 Waste tube or beaker

 9.4 HIC Buffers:

 9.4.1 Binding buffer (= 4.0M ammonium sulfate in TE Buffer pH 8)

 9.4.2 Equilibration buffer (= 2.0M ammonium sulfate in TE Buffer pH8)

 9.4.3 Wash buffer (= 1.3M ammonium sulfate in TE Buffer pH8)

 9.4.4 Elution buffer (= TE buffer)

 9.5 Test tube rack

 9.6 Magic marker

 9.7 3 test tubes (5 ml volume)
 9.8 disposable pipettes ~ 3 ml

9.9 Pipetman 1000ul with tips

9.10 UV black light

**10.0 Procedure (Illustrated Overview):**



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 10.1 Remove caps from the top and bottom of the HIC column and then allow the
 fluid within to drain.

 10.2 Add 2 ml of Equilibration buffer to the top of the column. Allow the column to drain into the waste beaker. Cap the bottom of the column.

10.3 Transfer 250µl of bacterial cell lysate to a 1.0ml microfuge tube and add 250µl of binding buffer to it.

 10.4. Label three 5ml plastic test tubes in sequence from 1-3.

 10.4.1 Place test tube number 1 under the column. Remove the cap

 from the bottom of the column and add 250 l of the bacterial cell lysate

 supernatant/ Binding buffer used in 10.3. to the top of the
 column. Allow the solution to drain completely into test tube 1.

10.4.2 Place test tube number 2 under the column. Add 250 l of Wash buffer. Allow the buffer to drain to the top of the column into test tube 2.

10.4.3 Place test tube number 3 under the column. Add 750 l of Elution buffer to the column. Allow the buffer to drain completely into test tube 3. This tube should have pure GFP in it. It can be seen glowing green under the long range UV lamp.

 10.5 Note the GFP positive test tube(s) and save for future analysis by SDS-PAGE.

 **Part III**

 **Ion Exchange (Anion Ion Exchange) Chromatography (IEX)**

**11.0 Materials:**

 11.1 250µl of bacterial cell lysate supernatant

11.2 Pre-packed MacroPrep High Q for Anion Exchange Chromatography column

 11.3 Waste tube or beaker

11.4 Anion Ion Exchange Chromatography buffers:

 11.4.1 Equilibration buffer (=50 mM Tris pH 8.3)

 11.4.2 Elution buffer 1 (50 mM Tris, pH 8.3, 130 mM NaCl)

 11.4.3 Elution buffer 2 (50 mM Tris, pH 8.3, 200 mM NaCl)

 11.4.4 Elution buffer 3 (50 mM Tris pH 8.3, 300 mM NaCl)

 11.4.5 Elution buffer 4 (50mM Tris pH 8.3, 500 mM NaCl)

 11.5 Test tube rack

 11.6 Marker

 11.7 6 test tubes (5 ml volume)
 11.8 Disposable pipettes ~ 3 ml

11.9 Pipetman 1000ul with tips

11.10 UV black light

 11.11 Lab marker

 11.12 Microfuge tube

**12. Procedure:**

12.1 Label 5, 5ml test tubes in sequence from 1-5.

 12.2 Remove cap from the top and then the bottom of the IEX column

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12.3 Equilibrate the column by adding 2ml of 50mM Tris pH 8.3. Collect and discard
 into the waste beaker.

12.4 Load 250µl of the bacterial cell lysate supernatant onto the top of the column

 and collect the flow through fraction in test tube 1. Examine with UV light.

 Save 10ul of the flow through in a microfuge tube for future use.

12.5 Immediately add 250ul of Elution Buffer 1 (50 mM Tris, pH 8.3, 130 mM NaCl) to the top of the column and collect the eluate in test tube 2. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.

12.6 Add 250ul of Elution Buffer 2 (50 mM Tris, pH 8.3, 200 mM NaCl) to the top of the column and collect the eluate in test tube 3. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.

12.7 Add 750ul of Elution Buffer 3 (50 mM Tris, pH 8.3, 300 mM NaCl) to the top of the column and collect the eluate in test tube 4. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.

12.8 Add 250ul of Elution Buffer 4 (50 mM Tris, pH 8.3, 500 mM NaCl) to the top of the column and collect the eluate in test tube 5. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.

12.9 Using the UV light select the test tube(s) containing GFP for analysis by

 SDS-PAGE as well as any additional samples that were collected.

**Part III**

**Ion Exchange (Cation Ion Exchange) Chromatography (IEX)**

**13.0 Materials**

 13.1 Bacterial lysate or previous isolated sample, 250 µl

 13.2 Pre-packed IEX column (cation) Macro-Prep High S

 13.3 Waste tube or beaker

 13.4 Cation Ion Exchange Chromatography buffers:

 13.4.1 Equilibration buffer ( = 50 mM Tris pH 8.3)

 13.4.2 Elution Buffer 1 (50mM Tris, pH 8.3, 130 mM NaCl)

 13.4.3 Elution Buffer 2 (50 mM Tris, pH 8.3, 200 mM NaCl)

 13.4.4 Elution Buffer 3 (50 mM Tris, pH 8.3, 300 mM NaCl)

 13.4.5 Elution Buffer 4 (50 mM Tris, pH 8.3, 500 mM NaCl)

13.5 Test tube rack

13.6 Marker

13.7 5 test tubes (5 ml volume)

13.8 Disposable pipettes ~3ml

13.9 Pipetman 1000 µl with tips

13.10 UV black light

13.11 Microfuge tube

**14.0 Procedure:**

14.1 Label 5 (5 ml) test tubes in sequence from 1-5 and place into test tube rack.

14.2 Remove cap from the top and then the bottom of the IEX column.

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14.3 Equilibrate the column by adding 2 ml of 50mM Tris pH 8.3. Collect and discard

 into a waste beaker.

14.4 Load 250 µl of the bacterial cell lysate supernatant onto the top of the column

 and collect the flow through fraction (eluate) into test tube 1. Examine with

 UV light; if GFP is present it will glow.

 Save 10 µl of flow through liquid in a microfuge tube for future use.

 14.5 Immediately add 250 µl of Elution Buffer 1 (50mM Tris, pH 8.3, 130mM NaCl)

 to the top of the column and collect the eluate in test tube 2. Examine with UV

 light again as in 14.4. Save 10 µl of the flow through liquid in a microfuge tube

 for future use.

14.6 Add 250 µl of Elution Buffer 2 (50mM Tris pH 8.3, 200mM NaCl) to the top of the column and collect the eluate in test tube 3. Save 10 µl of the flow through in a microfuge tube for future use.

14.7 Add 750 µl of Elution Buffer 3 (50 mM Tris pH 8.3, 300mM NaCl) to the top of the column and collect the eluate in test tube 4. Examine with UV light. Save 10 µl of the flow through in a microfuge tube for future use.

14.8 Add 250 µl of Elution Buffer 4 (50 mM Tris, pH 8.3, 500mM NaCl)to the top of the column and collect the eluate in test tube 5. Examine with UV light. Save 10 µl of the flow through to a microfuge tube for future use.

14.9 Using the UV light select the test tube(s) containing GFP for analysis by SDS-PAGE or include all samples if you wish.