Biotechnology Explorer™

Protein Expression and Purification Series

Planning Guide

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Catalog #166-5040EDU, Centrifugation Purification Process
Catalog #166-5045EDU, Hand-Packed Purification Process
Catalog #166-5050EDU, Prepacked Purification Process
Catalog #166-5070EDU, Assessment Module

Note: This document is for planning purposes only and may vary from the final product specifications.

For technical service, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.

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Overview

The intention of this planning guide is to provide an overview of the Protein Expression and Purification Series, including details of timelines, materials included, and equipment requirements, so that instructors may prepare their curriculum and estimate budgetary requirements in advance. These are estimates based on current information available, and may change in the final version of the lab series.

The Protein Expression and Purification Series comprises 4 lab modules with 3 purification options. The modular nature of the series allows you to choose from three different purification options depending on your teaching goals and equipment resources.

If you have further questions about the lab series, please contact biotechnology_explorer@bio-rad.com.

Protein Expression and Purification Series Components

The manual provided with the series comes with complete curricula for all three purification options. The hand-packed and prepacked purification processes require chromatography instrumentation.

Centrifugation Purification Process (catalog #166-5040EDU)

Growth and Expression Module
SDS-PAGE Electrophoresis Module
Centrifugation Purification Module
DHFR Enzymatic Assay Module

Hand-Packed Purification Process (catalog #166-5045EDU)

Growth and Expression Module
SDS-PAGE Electrophoresis Module
Hand-Packed Column Purification Module
DHFR Enzymatic Assay Module

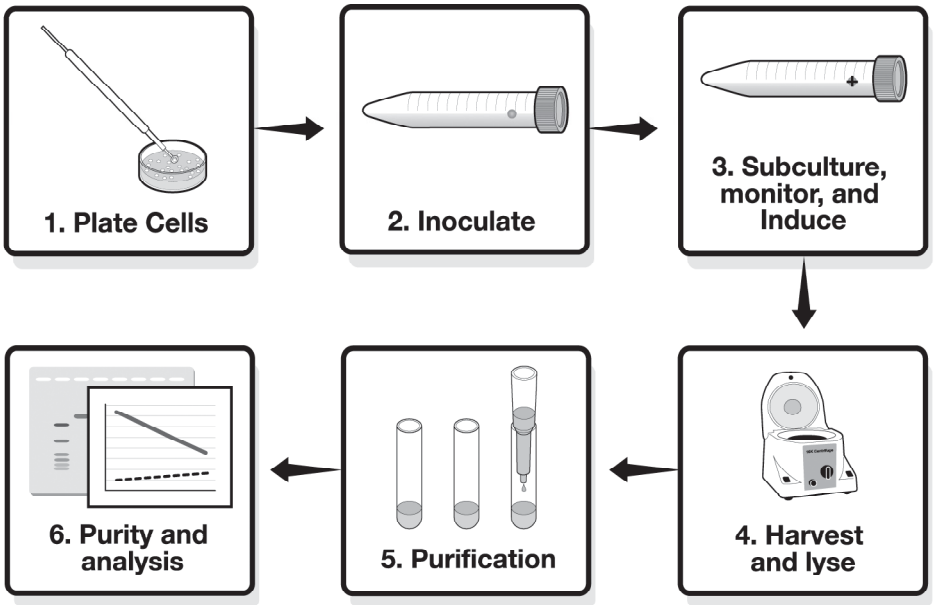
Prepacked Purification Process (catalog #166-5050EDU)

Growth and Expression Module
SDS-PAGE Electrophoresis Module
Prepacked Cartridge Purification Module
DHFR Enzymatic Assay Module

Assessment Module (catalog #166-5070EDU)

This unique assessment guide provides ideas for using formative assessment in your class to guide and increase learning while students perform the lab activities. At the end of the lab series use the summative assessment to evaluate the final learning levels of students. The assessment tool is arranged according to learning levels so that you can choose what best evaluates the comprehension of the students.

Series Overview:



Series Summary

The Protein Expression and Purification Series is a modular laboratory course designed to serve four to 12 student teams, depending on which purification option is used. The aim of this course is to express and purify His- and GST-tagged dihydrofolate reductase (GST-DHFR-His) and then analyze the isolated purified protein fractions for enzymatic activity.

This project involves growing cultures of GST-DHFR-His, lysing the bacterial cell cultures, separating soluble from insoluble fractions, purifying the GST-DHFR-His via centrifugation or protein purification instrumentation, analyzing protein purity via SDS-PAGE and analyzing protein enzymatic activity. DHFR is an essential protein used in the conversion of dihydrofolate (DHF) into tetrahydrofolate (THF) by the addition of a hydride from NADPH. THF is a methyl (CH_3) group shuttle required for synthesis of essential molecules such as nucleotides and amino acids. DHFR deficiencies are extreme and result in symptoms such as megaloblastic anemia and cerebral folate metabolism disorders. DHFR inhibition or reduction disrupts nucleic acid synthesis and cell growth, resulting in cell death.

This project offers an opportunity to perform true affinity purification with a realistic research workflow in which students can examine the enzymatic activity of the purified protein product. It also introduces students to the world of biomanufacturing where the research process is scaled up and standard operating procedures and work instructions are followed strictly during the production process.

Independent research extensions such as further purification steps, ELISA, western blotting, site-directed mutagenesis and many other options are possible once students have purified the protein.

The steps in this project to express and purify GST-DHFR-His are:

1. Understanding recombinant protein expression, DHFR, and protein purification.
2. Setting up the experiments.
3. Growing cultures.
4. Inducing expression of the GST-DHFR-His protein.
5. Lysing the bacterial cells to release the expressed protein.
6. Using SDS-PAGE analysis to verify expression of the protein, identify fractions containing purified protein, and to assess level of purity.
7. Purifing the GST-DHFR-His protein via affinity chromatography followed by desalting of the samples.
8. Analyzing the DHFR enzymatic activity.

Materials Required But Not Supplied

Chemicals	Centrifugation Purification	BioLogic™ LP/BioLogic DuoFlow™ Purification
Dry ice	1 block	1 block
Ethanol	0.5–2 L	0.5–2 L

Plastics and Consumables	Centrifugation Purification	BioLogic LP/BioLogic DuoFlow Purification
100–1,000 µl pipet tips, standard style (catalog #223-9350EDU)	12 boxes	4 boxes
2–200 µl pipet tips, standard style (catalog #223-9347EDU)	12 boxes	4 boxes
Parafilm sealing film	1	1
UV compatible cuvettes such as trUView™ cuvettes (catalog #170-2510EDU)	1 box of 50	1 box of 50
or	or	or
quartz submicrovolume cuvettes (catalog #170-2505EDU)	1–4 cuvettes	1–4 cuvettes
1.5 ml standard disposable polystyrene cuvettes (catalog #223-9955EDU)	1 box of 100	1 box of 100
or		or
quartz standard cuvettes (catalog #170-2502EDU)	1–4 cuvettes	1–4 cuvettes
10 ml syringes	12	16
22 gauge syringe needles	12	4
Fraction collection tubes (catalog #223-9751EDU)	N/A	200 tubes

Glassware	Centrifugation Purification	BioLogic LP/BioLogic DuoFlow Purification
500 ml Erlenmeyer flasks	N/A	4
1 L Erlenmeyer flask or autoclavable bottle	2	1
Beakers for dry ice/ethanol bath	1–12	1–4

Temperature Control Equipment and Mixing Devices	Centrifugation Purification	BioLogic LP/BioLogic DuoFlow Purification
Microwave oven	1	1
–20°C freezer	1	1
Incubation oven (catalog #166-0501EDU)	1–2*	1
Tube roller (catalog #166-0711EDU)	1–2*	N/A
Mini rocker (catalog #166-0710EDU)	1**	1
Shaking incubator or shaking water bath	1*	N/A
capable of holding 4 x 500 ml flasks	N/A	1
Dry bath (catalog #166-0562EDU) or water bath	1	1

* Initial overnight cell cultures and cell subcultures/induced cells for centrifugation purification are prepared in 50 ml sterile conical tubes. Incubation at 37°C with some form of mixing is required. This incubation can be accomplished in either a temperature-controlled shaking incubator, a shaking water bath, or using tube rollers in an incubation oven set at 37°C. The Bio-Rad tube roller can hold six 50 ml sterile conical tubes. Therefore, if 12 workstations are being run, two tube rollers, each in its own incubation oven, would be required.

** For the Centrifugation Purification protocol, during the binding of the cell lysate to the Profinity™ IMAC Ni-charged resin, a tube roller can be used for end over end mixing of the resin and lysate. If a tube roller is not available, the mixing can be accomplished by using a mini rocker. The mini rocker can be further used for rinsing, staining, and destaining of SDS-PAGE gels.

Other Equipment

	Centrifugation Purification	BioLogic LP/BioLogic DuoFlow Purification
UV/Vis Spectrophotometer capable of reading to three decimal places (catalog #170-2525EDU)	1	1
100–1,000 µl adjustable micropipet (catalog #166-0508EDU, 166-0553EDU)	12	4
20–200 µl adjustable micropipet (catalog #166-0507EDU, 166-0552EDU)	12	4
2–20 µl adjustable micropipet (catalog #166-0506EDU, 166-0551EDU)	12	4
Pipet pump or filler	12	4
10 ml serological pipets	4	12
Power supply (catalog #164-5050EDU)	3–12	1–4
Vertical electrophoresis chambers (catalog #165-8004EDU, 165-8005EDU)	3–12	1–4
Gel documentation system (catalog #170-8170EDU, 170-8270EDU)	1	1
Microcentrifuge with variable speed setting capable of 16,000 x g (catalog #166-0602EDU)	1	1
Centrifuge capable of 16,000 x g with rotors that hold 250 ml centrifuge bottles and 30–50 ml centrifuge tubes	N/A	1
Centrifuge bottles (250 ml) capable of withstanding 4,500 x g	N/A	4
Centrifuge tubes (30–50 ml) capable of withstanding 16,000 x g	N/A	4

Hand-Packed Column Process Only	Quantity
Flow adaptor, 1.0 cm column ID 1–7 cm functional length (catalog #738-0014EDU)	1 per instrument

Equipment Necessary If Using a BioLogic DuoFlow System (catalog #760-0047EDU or 760-0037EDU)

	Quantity
Fittings to convert luer to 1/4-28 (catalog #732-0113EDU)	1 set per instrument
5 ml injection loop (catalog #750-0497EDU)	1 per instrument

Miscellaneous

	Centrifugation Purification	BioLogic LP/BioLogic DuoFlow Purification
Marking pens	12	4
Storage boxes for microcentrifuge tubes (catalog #166-0482EDU)	3	1
Green racks for microcentrifuge tubes (catalog #166-0481EDU)	12	4

Optional Materials	Centrifugation Purification	BioLogic LP/BioLogic DuoFlow Purification
Autoclave	1	1
Vortexer (catalog #166-0610EDU)	1	N/A
Imaging system (catalog #170-8270EDU with 170-8272EDU)	1	1

Refills Available Separately

Each individual module is available to order as a stand alone product. In addition, certain refill items are also available:

Growth and Expression reagent pack, catalog #166-5057EDU, includes ampicillin (60 mg), LB agar pouch (20 g), LB capsules (12), lysozyme (25 mg), BL21(DE)3 with pDHFR, 1M IPTG (0.1 ml), sterile water (2.5 ml), 10x PBS (100 ml), imidazole stock solution (200 ml), and 20% sterile glucose solution (4 ml)

LB nutrient agar powder, 20 g, catalog #166-0600EDU

LB nutrient agar powder, 500 g, catalog #166-0472EDU

Ampicillin, 30 mg, catalog #166-0407EDU

10x PBS, 100 ml, catalog #166-2403EDU

Sterile water, 500 ml, catalog #163-2091EDU

Petri dishes, 60 mm, sterile, 500, catalog #166-0470EDU

Inoculation loops, 10 µl, sterile, 80, catalog #166-0471EDU

EZ Micro test tube, 2 ml, 500/box, catalog #223-9430EDU

1.5 ml conical tubes, with separate O-ring screwcaps, 500, catalog #224-0100EDU

1.5 ml conical tubes, with installed O-ring screwcaps, sterilized, 500, catalog #224-0110EDU

10x TGS buffer, 1 L, catalog #161-0732EDU

Laemmli sample buffer, 30 ml, catalog #161-0737EDU

Bio-Safe™ Coomassie stain, 1 L, catalog #161-0786EDU

Precision Plus Protein™ Dual Color standards, catalog #161-0374EDU

Jellyfish foam floating racks, 8 racks, catalog #166-0479EDU

Gel staining trays, 4, catalog #166-0477EDU

Profinity™ IMAC Ni-charged resin, 10 ml, catalog #156-0131EDU

1.0 cm x 5 cm, 2-pk Glass Econo-Column® columns, catalog #737-1007EDU

Micro Bio-Spin™ 6, Tris 25/pkg, catalog #732-6221EDU

Bio-Scale™ Mini cartridge, IMAC, 5 x 1 ml, catalog #732-4610EDU

Flow adaptor, 1.0 cm Column ID, catalog #738-0014EDU

Course Objectives

The Protein Expression and Purification Series is appropriate for the laboratory portion of an undergraduate (or early graduate) course in biotechnology, biochemistry, molecular biology, cell biology, recombinant DNA techniques, or advanced high school biotechnology-related courses. It would also be suitable for students doing independent research. It would be excellent for inclusion in biotechnology degree programs offered by community or technical colleges. The exercise could also prove useful for employers in the biotechnology, pharmaceutical, or industrial sectors as an introduction to or refresher on protein purification techniques, and particularly as an introduction to using chromatographic instrumentation.

Due to recent advances in the area of protein technology, the actual laboratory procedures are routine, safe, and relatively inexpensive, provided basic laboratory equipment is available. Measures have been taken to ensure the safety of the reagents used. While proper laboratory safety techniques must always be employed, the reagents provided are safe to use in the classroom. In order to complete the laboratory project in five to eight sessions, it is assumed that students meet at least once per week in a three-hour laboratory session, and that students can meet during the week to carry out a quick laboratory task or two.

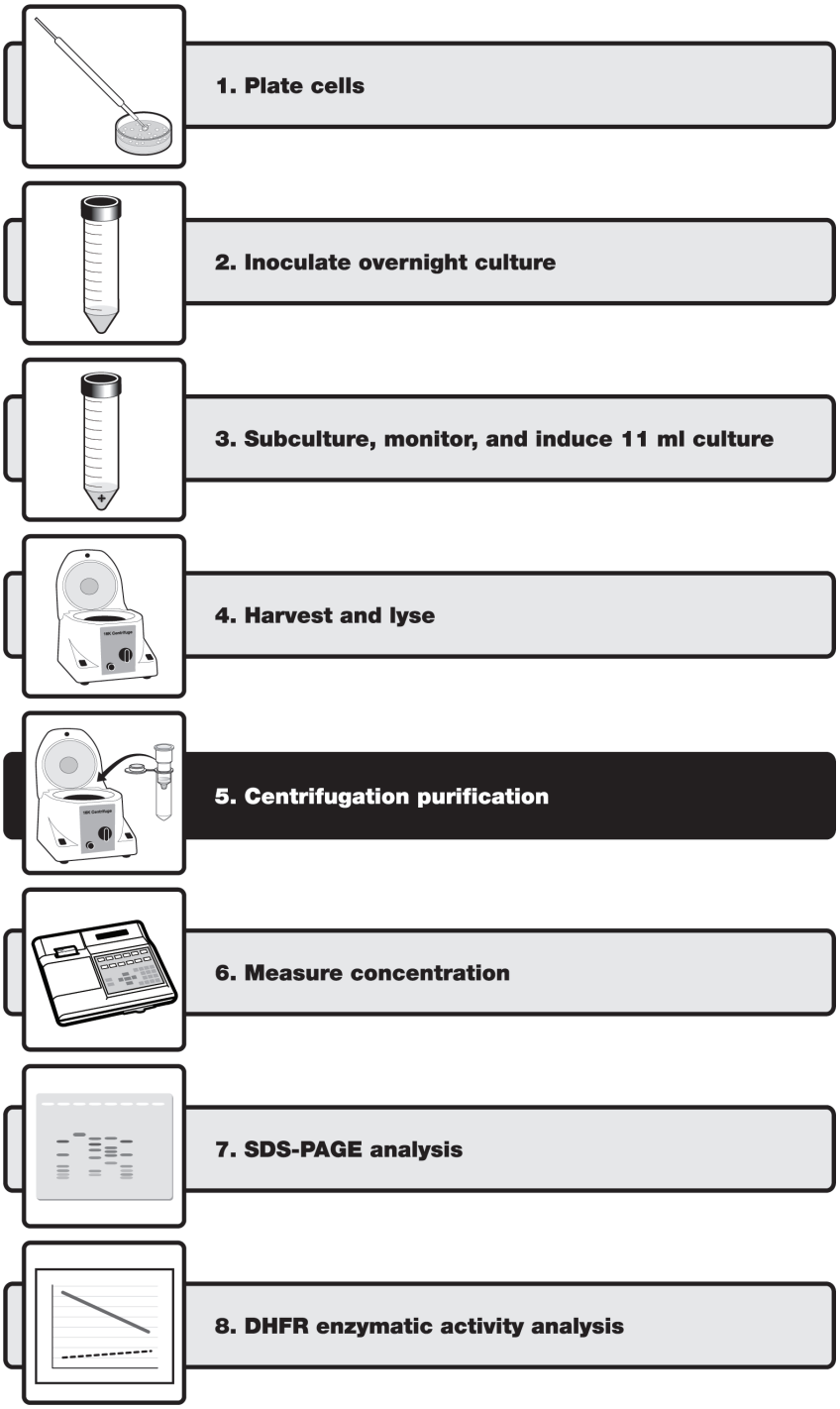
Specific Objectives Met by This Project

1. Students will experience a wide range of laboratory techniques. Some of the techniques implemented in this protein-based project are: cell culturing techniques, protein expression and purification, basic micropipetting, gel electrophoresis, and enzymatic analyses.
2. Students will see that these individual techniques are just steps in a longer investigatory process. Few researchers can complete an entire research project in one or two 3-hour laboratory sessions (the time frame of most commercially available kits), so this five- to eight-period project more accurately reflects what goes on in a contemporary molecular biology laboratory.
3. Students will be active participants in the process. There are numerous occasions during the project when students are asked to troubleshoot their results, or to make judgments about what to do next. This exercise does not take a simple “cookbook” approach, but rather it involves more critical thinking.

Timelines for the Laboratory Course

The timeline will depend greatly on the level of the students, which purification method is used, the length of class periods, and whether other techniques and analyses are performed in addition to the basic protocol. To assist in planning for the laboratory course, the following pages provide a workflow, timeline, and helpful hints guide for each purification process. The centrifugation purification process begins below, followed by the chromatography instrument purification process.

Centrifugation Purification Workflow



Centrifugation Purification Laboratory Timeline

Note: Tasks that are shaded in grey are preparatory tasks for later stages and may be conducted when spare time is available.

Centrifugation Purification				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
0	3A: Advanced Preparation for Centrifugation Purifiation Protocols	Pour LB/ampicillin (amp) plates	30 min	Growth and Expression
		Prepare LB and LB/amp broth	30 min	Growth and Expression
		Prepare Isopropyl beta-D-thiogalactopyranoside (IPTG)	5 min	Growth and Expression
1	4: Culturing, Expression, Lysis and SDS-PAGE Analysis for 11 ml Cultures and Purification Protocol for Centrifugation Process	Plating *E. coli*	15 min	Growth and Expression
		Grow *E. coli* plates overnight at 37°C	16+ hr*	Growth and Expression
2	4: Culturing, Expression, Lysis and SDS-PAGE Analysis for 11 ml Cultures and Purification Protocol for Centrifugation Process	Prepare overnight culture	15 min	N/A
		Grow overnight culture at 37°C	12+ hr*	N/A
3	4: Culturing, Expression, Lysis and SDS-PAGE Analysis for 11 ml Cultures and Purification Protocol for Centrifugation Process	Measure OD600 of overnight culture	30–45 min	N/A
		Prepare uninduced SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Prepare subcultures	15–30 min	Growth and Expression
		Grow subculture	1 hr	N/A
		Measure OD600 of subculture and induce with IPTG	30–45 min	Growth and Expression
		Culture induced cells at 37°C	4–24 hr*	N/A

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Centrifugation Purification continued				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
		Prepare equilibration buffer	5 min	Growth and Expression
		Prepare lysozyme	5 min	Growth and Expression
		Prepare lysis buffers	5 min	Growth and Expression
4	4: Culturing, Expression, Lysis and SDS-PAGE Analysis for 11 ml Cultures and Purification Protocol for Centrifugation Process	Prepare induced SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Pellet cells	30 min–1 hr	Growth and Expression
		Optional stopping point: Store pellet at –20°C to –80°C indefinitely		
		The remaining steps depend on the method chosen for performing lysis procedure:		
		Option 1: Dry ice/ethanol lysis *(recommended method)*		
		Resuspend cell pellet in lysis buffers	10 min	Growth and Expression
		Perform dry ice/ethanol lysis	30–45 min	Growth and Expression
		Optional stopping point: Store lysate at –20°C to –80°C indefinitely		
		Option 2: –70°C to –80°C lysis		
		Resuspend cell pellet in lysis buffers	10 min	Growth and Expression
		Place resuspended cells at –70°C to –80°C	16+ hr*	N/A
		Option 3: –20°C lysis		
		Resuspend cell pellet in lysis buffers	10 min	Growth and Expression

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Centrifugation Purification continued				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
		Place resuspended cells at –20°C	16+ hr*	N/A
		Thaw completely	30 min	N/A
		Place resuspended cells at –20° C	16+ hr*	N/A
5	4: Culturing, Expression, Lysis and SDS-PAGE Analysis for 11 ml Cultures and Purification Protocol for Centrifugation Process	Thaw lysate completely	30 min	N/A
		Separate soluble vs. insoluble fractions via centrifugation	20 min	N/A
		Decant soluble fraction	10 min	Growth and Expression
		Resuspend insoluble fraction in lysis buffer	15 min	Growth and Expression
		Prepare soluble and insoluble SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Optional stopping point: Store fractions at –20°C indefinitely		
6	4: Culturing, Expression, Lysis and SDS-PAGE Analysis for 11 ml Cultures and Purification Protocol for Centrifugation Process	Prepare Micro Bio-Spin™ column with Profinity™ IMAC Ni-charged resin	15–30 min	Growth and Expression and Centrifugation Purification
		Bind GST-DHFR-His soluble fraction to IMAC Ni-charged resin	20–30 min	N/A
		Collect flowthrough, wash, and eluate fractions	15–30 min	Growth and Expression and Centrifugation Purification
		Prepare flowthrough, wash and eluate SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Centrifugation Purification continued				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
		Optional stopping point: Store fractions at 4°C and SDS-PAGE samples at –20°C indefinitely		
		Prepare desalting columns and desalt eluate fraction	30 min	Centrifugation Purification
		Prepare desalted eluate SDS-PAGE sample	15 min	SDS-PAGE Electrophoresis
		Optional stopping point: Store fractions at 4°C and SDS-PAGE samples at –20°C indefinitely		
7	4 and 8: Culturing, Expression, Lysis and SDS-PAGE Analysis for 11 ml Cultures and Purification Protocol for Centrifugation Process; and DHFR Analysis	Reheat SDS-PAGE samples	15–30 min	N/A
		Electrophorese SDS-PAGE samples	0.5–1 hr	SDS-PAGE Electrophoresis
		Rinse and stain gels	1.25+ hr*	SDS-PAGE Electrophoresis
		Destain gels	8+ hr*	N/A
		Measure and calculate desalted eluate concentration	30 min	N/A
		Prepare enzymatic assay reagents *(Must be prepared within 3–4 hrs of use)*	15 min	DHFR Enzymatic Assay
		Perform enzymatic analysis	2 hr	Growth and Expression and DHFR Enzymatic Assay

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Centrifugation Purification Helpful Hints Checklist

Overnight Cultures

- 37°C incubation temperature of cultures is required.
- If using a shaking incubator or shaking water bath, a rotational speed of 250–275 rpm is required.
- If using a tube roller in an incubator, the tube roller should be in end over end mixing mode.

Subcultures

- Ensure the LB/amp medium is prewarmed to 37°C.
- 37°C incubation temperature of cultures is required.
- If using a shaking incubator or shaking water bath, a rotational speed of 250–275 rpm is requried.
- If using a tube roller in an incubator, the tube roller should be in end over end mixing mode.

Induction

- Determine timeline for induction. Four hour induction provides optimal results.
- 37°C incubation temperature of cultures is required.
- If using a shaking incubator or shaking water bath, a rotational speed of 250–275 rpm is required.
- If using a tube roller in an incubator, the tube roller should be in end over end mixing mode.

Cell Lysis

- Prepare lysozyme fresh (within 24 hours of use) for best results.
- Make sure lysozyme was made in 2x phosphate buffered saline (PBS).
- Make sure the cell pellets are completely resuspended in lysis buffer 1.

Separating Soluble and Insoluble Fractions

- The centrifuge must be able to generate 16,000 x g of force (relative centrifugal force, RCF). RCF is not equal to RPM. Make sure that the correct conversion from RCF to RPM is calculated. For more information on this conversion, please refer to Appendix C in the series instruction manual.

Centrifugation Affinity Purification

- The microcentrifuge must be able to generate 1,000 x g of force (RCF). RCF is not equal to RPM. Make sure that the correct conversion from RCF to RPM is calculated. For more information on this conversion, please refer to Appendix C in the series instruction manual.
- Do not use a mini-centrifuge for this procedure because they generally generate 2,000 x g (RCF), which is too much force for the affinity Ni-IMAC resin to withstand.
- Ensure the Ni-IMAC resin is fully resuspended before preparing columns.
- Ensure the sample binds to the resin for 20 minutes with rocking.

Size Exclusion Purification (Desalting)

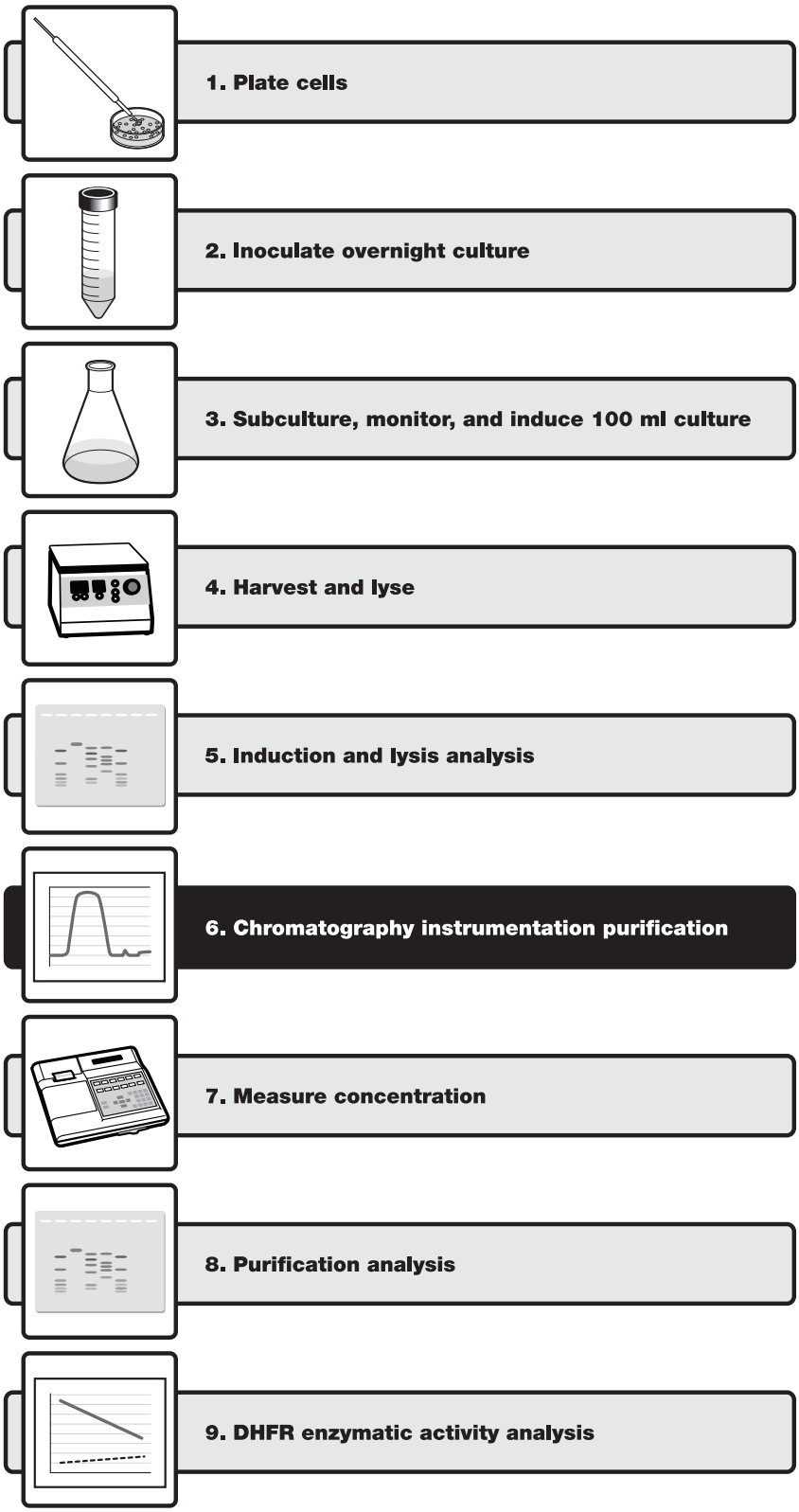
- The microcentrifuge must be able to generate 1,000 x g of force (RCF). RCF is not equal to RPM. Make sure that the correct conversion from RCF to RPM is calculated. For more information on this conversion, please refer to Appendix C in the series instruction manual.
- Do not use a mini-centrifuge for this procedure because they generally generate 2,000 x g (RCF), which is too much force for the Bio-Gel® P6 desalting resin to withstand.
- Removal of the storage buffer from the desalting columns is important. Make sure to let the resin drain via gravity to remove the first portion of the storage buffer.
- Make sure to use 2 ml microcentrifuge tubes during the desalting process. 1.5 ml microcentrifuge tubes are not large enough to allow the buffer to flow from the column into the collection tube.
- Verify that the desalted sample volume is not >150 µl (assuming two desalting runs using the same column). If the volume is >150 µl, then the column storage buffer was likely not removed properly.

DHFR Enzymatic Assay

- Ensure the dihydrofolate (DHF) is reconstituted in 10x PBS.
- Ensure the dihydronicotinamide-adenine dinucleotide phosphate (NADPH) is reconstituted in 1x PBS.
- Ensure the DHF and NADPH are used within three to four hours. The reconstituted DHF and NADPH cannot be frozen to retain or extend the activity and shelf life.
- The enzymatic reaction occurs very quickly, so make sure to add the DHF to the NADPH solution and then place it immediately in the spectrophotometer. It is best to do this step next to the spectrophotometer to ensure no data points are missed.
- Make sure the spectrophotometer is turned on at least 30 minutes ahead of time to allow the lamp to warm up.
- If using trUView™ cuvettes, make sure the frosted side is facing the beam path. The frosted side of the trUView cuvettes contains a tiny clear window through which the UV reading is taken.

Chromatography Instrumentation Purification Workflow

The timeline will depend greatly on the level of the students, the length of class periods, and whether other techniques and analyses are performed in addition to the basic protocol.



Chromatography Instrumentation Purification Laboratory Timeline

Note: Tasks that are shaded in grey are preparatory tasks for later stages and may be conducted when spare time is available.

Chromatography Instrumentation Purification				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
0	3B: Advance Preparation—Instrumentation Protocols	Pour LB/ampicillin (amp) plates	30 min	Growth and Expression
		Prepare LB and LB/amp broth	30 min	Growth and Expression
		Prepare instrumentation	2+ hr*	N/A
		Optional: Run BioLogic starter kit	2+ hr*	N/A
1	5: Culturing, Expression, Lysis and SDS-PAGE Anlaysis for 100 ml Cultures	Plate *E. coli*	15 min	Growth and Expression
		Grow *E. coli* plates overnight at 37°C	16+ hr*	Growth and Expression
2	5: Culturing, Expression, Lysis and SDS-PAGE Anlaysis for 100 ml Cultures	Prepare overnight culture	15 min	N/A
		Grow overnight culture at 37°C	12+ hr*	N/A
3	5: Culturing, Expression, Lysis and SDS-PAGE Anlaysis for 100 ml Cultures	Measure OD600 of overnight culture	30–45 min	N/A
		Prepare uninduced SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Prepare subcultures	15–30 min	Growth and Expression
		Grow subcultures	1 hr	N/A
		Measure OD600 of subculture and induce with IPTG	30–45 min	Growth and Expression
		Culture induced cells at 37°C	4–24 hr*	N/A

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Chromatography Instrumentation Purification continued				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
		Prepare equilibration buffer	5 min	Growth and Expression
		Prepare lysozyme	5 min	Growth and Expression
		Prepare lysis buffer	5 min	Growth and Expression
4	5: Culturing, Expression, Lysis and SDS-PAGE Analysis for 100 ml Cultures	Prepare induced SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Pellet cells	0.5–1 hr	Growth and Expression
		Optional stopping point: Store pellet at –20°C to –80°C indefinitely		
		The remaining steps depend on method chosen for performing lysis procedure:		
		Option 1: Dry ice/ Ethanol lysis *(recommended method)*		
		Resuspend cell pellet in equilibration and lysis buffer	10 min	Growth and Expression
		Perform dry ice/ethanol lysis	30–45 min	Growth and Expression
		Optional stopping point: Store lysate at –20°C to –80 °C indefinitely		
		Option 2: –70°C to –80°C lysis		
		Resuspend cell pellet in equilibration and lysis buffer	10 min	Growth and Expression
		Place resuspended cells at –70°C to –80°C	16+ hr*	N/A
		Option 3: –20°C lysis		
		Resuspend cell pellet in equilibration and lysis buffer	10 min	Growth and Expression

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Chromatography Instrumentation Purification continued				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
		Place resuspended cells at –20°C	16+ hr*	N/A
		Thaw completely	30 min	N/A
		Place resuspended cells at –20°C	16+ hr*	N/A
5	5: Culturing, Expression, Lysis and SDS-PAGE Analysis for 100 ml Cultures	Thaw lysate completely	30 min	N/A
		Separate soluble vs. insoluble fractions via centrifugation	20 min	N/A
		Decant soluble fraction	10 min	Growth and Expression
		Resuspend insoluble fraction in equilibration buffer	15 min	Growth and Expression
		Prepare soluble and insoluble SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Optional stopping point: Store fractions at –20°C indefinitely		
		Reheat uninduced, induced, soluble, and insoluble SDS-PAGE samples	10 min	N/A
		Electrophorese SDS-PAGE samples	0.5–1 hr	SDS-PAGE Electrophoresis
		Rinse and stain gels	1.25+ hr*	SDS-PAGE Electrophoresis
		Destain gels	8+ hr*	N/A
6	5: Culturing, Expression, Lysis and SDS-PAGE Analysis for 100 ml Cultures	Optional: Prepare hand-packed Profinity IMAC Ni-charged columns	0.5–1 hr	Hand-Packed Column Purification Module

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Chromatography Instrumentation Purification continued				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
	6: Purification Protocol for BioLogic LP System or 7: Purification Protocol using BioLogic DuoFlow System (Depending on instrument used)	Perform chromatographic separation using chromatography instrumentation	3–4 hr	Growth and Expression and Hand-Packed Column Purification Module or Prepacked Cartridge Purification Module
		Optional stopping point: Store all fractions at 4°C		
7	6: Purification Protocol for BioLogic LP System or 7: Purification Protocol using BioLogic DuoFlow System (Depending on instrument used)	Study chromatogram and determine correct fractions for further analysis	30 min	N/A
		Prepare flowthrough, wash, and 3 eluate fractions SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Prepare desalting columns and desalt eluate fraction	30 min	Hand-Packed Column Purification Module or Prepacked Cartridge Purification Module
		Prepare desalted eluates, SDS-PAGE samples	15 min	SDS-PAGE Electrophoresis
		Optional stopping point: Store fractions at 4°C and SDS-PAGE samples at –20°C (reheat samples prior to electrophoresis if stored as noted)		
		Electrophorese SDS-PAGE samples	0.5–1 hr	SDS-PAGE Electrophoresis
		Rinse and stain gels	1.25+ hr*	SDS-PAGE Electrophoresis
		Destain gels	8+ hr*	N/A

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Chromatography Instrumentation Purification continued				
Lab Session	Chapter	Task	Estimated Duration	Module Containing Materials
		Meaure and calculate desalted eluate concentration	30 min	N/A
		Prepare enzymatic assay reagents *(Must be prepared within 3–4 hrs of use)*	15 min	DHFR Enzymatic Assay
		Perform enzymatic analysis	2 hr	Growth and Expression and DHFR Enzymatic Assay

*Time indicated for these tasks is not hands-on time. It is the time needed for reactions to run, bacteria to grow, or incubation to occur.

Chromatography Instrumentation Purification Helpful Hints Checklist

Overnight Cultures

- 37°C incubation temperature of cultures is required.
- If using a shaking incubator or shaking water bath, a rotational speed of 250–275 rpm is required.
- If using a tube roller in an incubator, the tube roller should be in end over end mixing mode.

Subcultures

- Ensure the LB/amp medium is prewarmed to 37°C.
- 37°C incubation temperature of cultures is required.
- If using a shaking incubator or shaking water bath, a rotational speed of 250–275 rpm is required.
- If using a tube roller in an incubator, the tube roller should be in end over end mixing mode.

Induction

- Determine timeline for induction. Four hour induction provides optimal reqults.
- 37°C incubation temperature of cultures is required.
- If using a shaking incubator or shaking water bath a rotational speed of 250–275 rpm is required.
- If using a tube roller in an incubator, the tube roller should be in end over end mixing mode.

Cell Lysis

- Prepare lysozyme fresh (within 24 hours of use) for best results.
- Make sure lysozyme was made in 2x phosphate buffered saline (PBS).
- Make sure the cell pellets are completely resuspended in lysis buffer.

Separating Soluble and Insoluble Fractions

- The centrifuge must be able to generate 16,000 x g of force (relative centrifugal force, RCF). RCF is not equal to RPM. Make sure that the correct conversion from RCF to RPM is calculated. For more information on this conversion, please refer to Appendix C in the series instruction manual.

Chromatography Instrumentation Affinity Purification

BioLogic LP System

- Run through the instrument's starter kit prior to performing the Protein Expression and Purification Series to ensure that both the wiring and tubing were installed correctly.
- Remember to calculate the dead volume (typically 2–4 ml) when determining which fractions contain the eluted GST-DHFR-His.

BioLogic DuoFlow System

- Run through the instrument's starter kit prior to performing the Protein Expression and Purification Series to ensure that both the wiring and tubing were installed correctly.

Size Exclusion Purification (Desalting)

- The microcentrifuge must be able to generate 1,000 x g of force (RCF). RCF is not equal to RPM. Make sure that the correct conversion from RCF to RPM is calculated. For more information on this conversion, please refer to Appendix C in the series instruction manual.
- Do not use a mini-centrifuge for this procedure because they generally generate 2,000 x g (RCF), which is too much force for the Bio-Gel® P6 desalting resin to withstand.
- Removal of the storage buffer from the desalting columns is important. Make sure to let the resin drain via gravity to remove the first portion of the storage buffer.

- Make sure to use the 2 ml microcentrifuge tubes during the desalting process. 1.5 ml micro-centrifuge tubes are not large enough to allow the buffer to flow from the column into the collection tube.
- Verify that the desalted sample volume is not >150 µl (assuming two desalting runs using the same column). If the volume is >150 µl, then the column storage buffer was likely not removed properly.

DHFR Enzymatic Assay

- Ensure the dihydrofolate (DHF) is reconstituted in 10x PBS.
- Ensure the dihydronicotinamide-adenine dinucleotide phosphate (NADPH) is reconstituted in 1x PBS.
- Ensure the DHF and NADPH are used within three to four hours. The reconstituted DHF and NADPH cannot be frozen to retain or extend the activity and shelf life.
- The enzymatic reaction occurs very quickly, so make sure to add the DHF to the NADPH solution and then place it immediately in the spectrophotometer. It is best to do this step next to the spectrophotometer to ensure no data points are missed.
- Make sure the spectrophotometer is turned on at least 30 minutes ahead of time to allow the lamp to warm up.
- If using trUView™ cuvettes, make sure the frosted side is facing the beam path. The frosted side of the trUView cuvettes contains a tiny clear window through which the UV reading is taken.

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