



A project funded by the California Community College Chancellor's Office to serve the employment and educational needs of the biotechnology community of Southern California.

Non-radioactive colony hybridization to detect a foreign protein.

**Laboratory protocol from the
Southern California Biotechnology Center.
For more information, please contact us at (760) 795-6648**

Purpose:

The specificity of antibody-antigen interactions will be used to detect those bacterial cells in a mixed population that produce a foreign protein. This unit provides exposure to sterile technique, serial dilution, replica plating of bacterial cells, membrane immobilization of cellular products, antibody binding and specificity and enzyme-linked substrates and assays.

Skills and content areas contained in the Non-radioactive Colony Hybridization to Detect Foreign Proteins

- Use of micropipettors
- Sterile technique
- Serial dilutions
- Replica plating of bacterial colonies
- Immobilization of proteins on membrane supports
- handling of membranes and antibodies
- enzyme-based colorimetric identification of antibody-antigen interactions

- "Western" hybridization to identify proteins

Materials: *(If you reside within our service area, materials and training to assist you to perform the procedure are available from the Southern California Biotechnology Center)*

- Stock LBamp plate of *E. coli* containing pBS and pFKBP
- LBampIPTG agar plates (4 per student)
- LBamp agar plates (1 per student)
- Nitrocellulose 82 mm membrane
- 20 mg/ml lysozyme stock solution
- Bovine Serum Albumin
- 10 X PBS
- Tween-20 detergent
- nonfat dry milk
- 3 ul Primary antibody [α -FKBP, r 77]
- 1.5 ul Secondary antibody [Goat anti-rabbit alkaline phosphase conjugate]
- Color detection reagents: 66 ul NBT, 33 ul BCIP per student

Other required materials and supplies:

- Pipettes and pipette bulb
- Glass rod spreaders, tape, strikers, marking pens, bunsen burners
- Alcohol (95%) in beakers or jars to flame spreaders
- 15 ml culture tubes for making serial dilutions



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| (5 per student) | optional |
| ➤ Sterile saline (~25 ml per student) for making dilution series. | ➤ Plastic storage box with lid |
| ➤ India ink (in small, short tube) and small gauge needle | ➤ Various graduated cylinders (500, 100, 50) |
| ➤ forceps | ➤ 1 M Tris-HCl, pH 8.0 |
| ➤ Chloroform, large beaker, and clamps - | ➤ 1 M Tris-HCl, pH 9.5 |
| | ➤ 5 M NaCl |
| | ➤ 1 M MgCl ₂ |

Egg White Lysozyme: Purification and Assay by Ion Exchange Chromatography

**Laboratory Activity from the
Southern California Biotechnology Center
For more information, please contact us at (760) 795-6648**

Purpose:

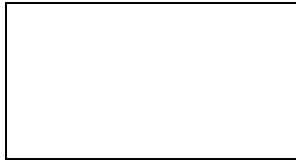
Lysozyme, a protein enzyme, will be purified from chicken egg whites using ion exchange column chromatography. Basic principles of column chromatography, general concepts in protein purification (such as yield and specific activity), the process of data collection, and the fundamentals of end-point and rate determination assays will be introduced. This unit build on and complements a similar unit in protein purification of lysozyme using size exclusion chromatography that is available from the Southern California Biotechnology Center.

Materials: *(If you reside within our service area, materials and training to assist you to perform the procedure are available from the Southern California Biotechnology Center)*

- CM Sephadex 25 chromatography resin
- 5 or 10 ml syringe barrels, or cut off pipettes, to be used as columns
- 10 ml syringe to be used as a filtering unit
- Carbonate buffer stock (1 M NaHCO₃, pH 10.5)
- Lyophilized *M. luteus* cells
- Bradford Protein Dye Reagent
- Bovine Serum Albumin protein standard
- Beta-mercaptoethanol

Other required materials and supplies:

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| ➤ Large chicken egg (one per student pair) | ➤ 1 M Tris-HCl, pH 8.2 |
| ➤ 5 M NaCl | ➤ 1 M Tris-HCl, pH 9 |



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- 1 M KH_2PO_4
- 1 M K_2HPO_4
- Spectrophotometer, visible range, and cuvettes
- Rocking device (optional, you may just occasionally mix by hand)
- Cheesecloth
- Ice bucket
- Glass wool
- Tubing and clamp for bottom of column
- Ring stand and clamp to hold column upright
- 50 and 100 ml beakers
- Small tubes to collect 0.5 ml column fractions (~30 per column)
- Large test tube for preparation of egg white extract



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Egg White Lysozyme : Purification and Assay by Size Exclusion Chromatography

Laboratory activity from
the Southern California Biotechnology Center.
For more information, please contact us at (760) 795-6648

Purpose:

Lysozyme, a protein enzyme, will be purified using size exclusion column chromatography from chicken egg whites. Basic principals of column chromatography, general concepts in protein purification (such as yield and specific activity), the process of data collection, and the fundamentals of end-point and rate determination assays will be introduced.

Skills and content areas contained in Size Exclusion Chromatography

- Solution preparation
 - Handling Biogel P20 chromatography resin (swelling, defining)
- Pouring a gel filtration column, applying samples and collecting fractions
- Calibrating a size exclusion column with colored protein standards
- Using spectrophotometers
- Setting up assays and collecting data
- rate assay (*M. luteus* lysis for lysozyme activity)
- end-point determination assay (Bradford dye binding for total protein concentration)
- Mathematically calculating values such as **yield** and **specific activity** of a purified protein

Materials: (If you reside within our service area, materials and training to assist you to perform the procedure are available from the Southern California Biotechnology Center)

- Biogel P20 chromatography resin
- Beta-mercaptoethanol (optional)
- Colored protein standards mix [1:1:1:1 mix of blue dextran, Cytochrome C and vitamin B12 at 10 mg/ ml each]
- 10 ml syringe barrel with glass wool plug and collection tube
- Lyophilized *M. luteus cells*
- Bradford Protein Dye Reagent
- Bovine Serum Albumin protein standard
- Large chicken egg (one per student pair)
- 5 M NaCl
- 1 M Tris-HCl, pH 9
- 1 M KH_2PO_4
- 1 M K_2HPO_4
- Spectrophotometer, visible range, and cuvettes
- Graph paper
- Rocking device
- Cheesecloth
- Ice bucket
- 10 ml glass or plastic pipettes with tops cut off



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- Glass wool
- Tubing and clamp for bottom of column
- Ring stand and clamp to hold column upright
- 50 and 100 ml beakers
- Small tubes to collect 0.5 ml column fractions (~30 per column)
- Large test tube for preparation of egg white extract



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Easy Classroom PCR

**Laboratory protocol from the
Southern California Biotechnology Center.**

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Purpose:

This activity is designed to demonstrate the power of the polymerase chain reaction (PCR) to amplify the quantity of DNA in a sample. New DNA molecules will be created in the test tube by combining a DNA template, nucleotide substrates and polymerase enzyme. Students will observe the outcome of this process by agarose gel electrophoresis.

Materials: *(If you reside within our service area, materials and training to assist you to perform the procedure are available from the Southern California Biotechnology Center)*

- PCR reaction buffer
- Primers DNAs (#6600 and #6601) for PCR
- Nucleotide substrates
- Target DNA for amplification (pEF-Bos 213)
- Thermophilic DNA polymerase
- 0.5 ml microfuge tubes
- Parafilm
- Loading dye for agarose gels

Other required materials and supplies:

- 100 mM MgCl₂
- Mineral oil
- p20 pipetmen
- Water baths set to 60, 72, and 95 C (or pans/beakers of water on hot plates, or electric frying pans containing water)
- Gel rigs and power supplies for horizontal gel electrophoresis
- Agarose
- TBE buffer (Tris, borate, EDTA)
- Ethidium bromide or methylene blue to stain gels for visualization of DNA
- Stop watches or timers



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Enzyme-linked immunosorbant assay (ELISA)

Laboratory protocol from the Southern California Biotechnology Center.

For more information, please contact us at (760) 795-6648

Purpose:

This activity is designed to demonstrate one of the most powerful of all immunochemical techniques, the ELISA. This assay, in its many variations, utilizes the inherent attraction of protein molecules to plastic and the specificity of antibody-antigen interactions as a means of immobilizing antibodies in solution for quantitation. Such an assay might commonly be used to identify and quantify the amount of antibody in a blood serum sample.

Materials: *(If you reside within our service area, materials and training to assist you to perform the procedure are available from the Southern California Biotechnology Center)*

- Plastic multi-well plates
- Parafilm
- 10 x PBS
- 10 x PBST
- 2 % gelatin (Store at 4 C to prevent growth of mold)
- Antigen: 1.5 mg per ml bovine serum albumin. Store at 4 C.
- Primary antibody: 1:10 diluted rabbit anti-BSA. Store at 4 C.
- Unknown "patient" samples A, B, C, D
- Secondary antibody: 1:10 diluted horseradish peroxidase (HRP) coupled goat anti- rabbit IgG. Store at 4 C.
- HRP substrate: ABTS (Store at 4 C.)

Other required materials and supplies:

- Pipetmen (p200) or other device to allow transfer of 50 ul (or one drop) solution.
- Incubator or water bath, set to 37 C.