Protein Electrophoresis

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| Grade Level: | **11 & 12****Summer Intern** | Subject: | Biotechnology / Molecular Biology/ Protein Analysis/ Techniques | Prepared By: | **Larry Cosenza****C2 Biotechnologies, LLC****lcosenza@c2biotechnologies.com** |

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| Overview & PurposeLearn how to analyze total protein in a system. C2B uses this technique to follow recombinant protein expression. | Education Standards Addressed |

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|  | Teacher Guide | Student Guide |  |
| Objectives(Specify skills/information that will be learned.) | Time managementChemical safety, MSDSPippettingSample handling | Assembly gel apparatusCast polyacrylamide gelsPrepare samplesElectrophoreses samplesVisualize outcome | Materials Needed* SOPs
* MSDS sheets
* Chemical & Reagents
* Equipment
 |
| Information(Give and/or demonstrate necessary information) | Standard Operating Protocol (SOP)Chemical SafetyElectrophoresis theory | Gels polymerizeElectrophoreses protein marker alongside experimental sample. |
| Verification(Steps to check for student understanding) | Mock spill liquid acrylamide. What are the molecular weights of proteins in experimental sample | Consult MSDS – contain and clean spill-record event. Laboratory notebook-image of gel after electrophoresis and protein staining. List molecular weights of proteins in sample. | Other Resources<http://en.wikipedia.org/wiki/SDS-PAGE><http://www.bio.davidson.edu/courses/genomics/method/SDSPAGE/SDSPAGE.html>Molecular Cloning: A Laboratory Manual, Third Edition (3 Volume Set) [Paperback], Author: [Joe Sambrook](http://www.amazon.com/s/ref%3Dntt_athr_dp_sr_1?_encoding=UTF8&sort=relevancerank&search-alias=books&field-author=Joe%20Sambrook) |
| Activity(Describe the independent activity to reinforce this lesson) | Homework: SDS-PAGE analysis images of time course for recombinant protein expression.  | Answer questions about images. |
| Summary | Technical and analytic skills:Pippetting, analysis, chemical safety | SDS-PAGE analysis | Additional Notes |

**Estimated Costs for Reagents and Equipment**

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| **Vendor** | **Catolog #** | **Item** | **Quantity** | **Cost** | **Purpose** |
| Sigma | A4418 | ammonium sulfate | 100g | $14.20 | Protein electrophoresis |
| Sigma | W4502-1L | Water, distilled,deionized | 1L | $38.30 | Protein electrophoresis |
| Sigma | A3678-25g | Ammonium Persulfate | 25g | $19.50 | Protein electrophoresis |
| Sigma | G5516-100ML | Glycerol | 100 ml | $31.20 | Protein electrophoresis |
| Sigma | T9281-25ml | TEMED | 25 | $27.90 | Protein electrophoresis |
| Sigma | 50046 | Glycine | 1kg | $191.00 | Protein electrophoresis |
| Sigma | B7920 | Coomassie Brillant Blue R250 | 10g | $39.80 | Protein electrophoresis |
| Sigma | L4522-100ML | Sodium Dodecyl Sulfate (SDS) 10% Solution | 100ml | $35.60 | Protein electrophoresis |
| Sigma | A3574-100ML | Acrylamide/Bis-acrylamide 30% Solution | 100ml | $33.80 | Protein electrophoresis |
| Sigma | A3574 | Acrylamide/Bis-acrylamide 30% Solution | 5x100ml | $80.20 | Protein electrophoresis |
| Sigma | F5415-25ML | Ficoll (Type 400) | 25 ml | $30.40 | Protein electrophoresis |
| Sigma | 318744 | Bromphenol Blue Solution | 500ml | $38.40 | Protein electrophoresis |
| Sigma | X4126-10G | Xylene Cyanol | 10g | $40.80 | Protein electrophoresis |
| Sigma | M3641-1L | Methanol | 1L | $30.40 | Protein electrophoresis |
| Sigma | A6283 | Glacial Acetic Acid | 500 ml | $35.70 | Protein electrophoresis |
| Sigma | T4661 | Trizma | 1kg | $193.50 | Protein electrophoresis |
| NEB | P7711S | ColorPlus Prestained Protein Ladder | 1 tube | $95.00 |  |
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| BioRad | 165-8025 | Gel Electrophoresis System with power supply | 1 | $1,098.00 | Equipment |
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|  |  |  |  | **$2,073.70** | **Total Cost** |

**Estimated Time**

1. Casting gels 1 hr
2. Sample Preparation 10 minutes
3. Loading gel 10 minutes
4. Electrophoresis 1 hr
5. Staining gels 10 minutes
6. De-staining gels 30 minutes



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| SOP-100 |  |

SDS – PAGE Analysis

Procedure for resolving protein molecules in polyacrylamide gels.

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| Section | Materials and Reagents |
| 1 |

1.5 M Tris-HCl (pH 8.8)

Tris base 181.7 g in 1 L H2O, adjust pH to 8.8 using HCl.

0.5 M Tris-HCl (pH 6.8)

Tris base 60.6 g in 1L H2O, adjust pH to 6.8 using HCl.

10 % (w/v) SDS

SDS (sodium dodecal sulfate) 100g in 1L H2O.

2X SDS Sample Buffer

0.25 M Tris Hcl (pH 6.8)

4 % (w/v) SDS

20 % (v/v) glycerol

trace bromphenol blue

Note: For reducing conditions use 950 ul 2x SDS sample buffer plus 50 ul 2-mercaptoethanol.

10 % (w/v) Ammonium Persulfate (APS)

10 g ammonium sulfate in H2O, aliquot into 1 ml vials and store at –20 C until needed.

TEMED

Acrylamide/bisacrylamide stock solution

For 1 L

30 % (w/v) acrylamide 300 g

0.8 % (w/v) bisacrylamide 8 g

Add H2O to final volume and filter through 0.22 um membrane. Store at 4 C in the dark.

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| Section | Protocol |
| 2 |

Preparing the sample

Add equal volume of 2 x SDS sample buffer to sample. Heat in boiling water bath (100 C) for 5 min. Cool samples to room temperature and give brief spin to pellet insoluble material.

1. Casting the gels.

Wash all glassware. Assemble gel apparatus. Mix the lower resolving gel:

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| Lowere Resolving gel | > 50 kD | 30-40 kD | 20 –30 kD | <20 kD |
| % Acrylamide | 8% | 10 % | 12 % | 15 % |
| Acrylamide / bisacylamide solution | 2.13 ml | 2.67 ml | 3.20 ml | 4.00 ml |
| H2O | 3.87 ml | 3.33 ml | 2.80 ml | 2.00 ml |
| 1.5 M Tris-HCl (pH 8.8) | 2.00 ml | 2.00 ml | 2.00 ml | 2.00 ml |
| 10 % SDS | 80 ul | 80 ul | 80 ul | 80 ul |
| 10 % APS | 45 ul | 45 ul | 45 ul | 45 ul |
| TEMED | 12 ul | 12 ul | 12 ul | 12 ul |
| Final Volume | 8 ml | 8 ml | 8 ml | 8 ml |

Add the APS and TEMED last, mix gently but quickly. Pour solution into casting apparatus until height of liquid is about 1 cm from the top of the gel plates. Overlay with H2O and allow to polymerize for about 1 hr.

Prepare stacking gel:

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| Stacking Gel | 3 % |
| Acrylamide / bisacylamide solution | 0.75 ml |
| H2O | 3.00 ml |
| 0.5 M Tris-HCl (pH 6.8) | 1.25 ml |
| 10 % SDS | 50 ul |
| 10 % APS | 30 ul |
| TEMED | 8 ul |
| Final Volume | 5.00 ml |

After the resolving gel has polymerized pour off the upper liquid. Rinse the upper portion of the gel with water. Rinse quickly with stacking gel solution and then fill uper chamber with stacking gel solution. Insert comb between plates. The upper gel should polymerize in about ten minutes.

1. Load the protein samples

Mount plates into electrophoresis chamber , add running buffer and then remove comb from gels.

Load the protein samples into the wells through the running buffer. Take care that the samples do not cross contaminate wells.

1. Electrophorese the gel.

Attach the electric leads to the power supply, black to negative and red to the positive pole. Proteins bound with SDS are negatively charged at pH 8.3 and will migrate toward the positive electrode.

When the bromphenol blue enters the resolving gel increase the current to 18 mA per gel. Stop the power when the bromphenol blue reaches the bottom of the resolving gel. Do not let the dye run out of the bottom.

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| Section | References |
| 3 |

Molecular Cloning: A Laboratory Manual, Third Edition (3 Volume Set) [Paperback], Author: [Joe Sambrook](http://www.amazon.com/s/ref%3Dntt_athr_dp_sr_1?_encoding=UTF8&sort=relevancerank&search-alias=books&field-author=Joe%20Sambrook)



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Coomassie Blue Staining of Polyacrylamide Gels

Procedure for detecting proteins in polyacrylamide gels.

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| Section | Materials and Reagents |
| 1 |

Coomassie Brilliant Blue Staining Solution

(1 liter)

Coomassie Brilliant Blue R250 2.5 g

Methanol 400 ml

Glacial Acetic Acid 100 ml

H2O 500 ml

High Methanol Destain Solution

(1 liter)

Methanol 400 ml

Glacial Acetic Acid 100 ml

H2O 500 ml

4 % (v/v) Glycerol Solution

(1 liter)

Glycerol 40 ml

H2O 960 ml

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| Section | Protocol |
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1. Stain the gel in approximately 100 ml of staining solution. Incubate at room temperature on a rotating platform for 20 min.
2. Remove the staining solution. Used solution can be kept and reused until quality of staining decreases.
3. Rinse gel in destain solution. Remove excess stain by incubating gel in 100 ml of destain solution for 2 – 3 hr. destaining is complete when the background is essentially clear.
4. Equilibrate gel in 4 % glycerol for 1 hr. The glycerol helps to keep gel from cracking when dried.
5. Record results electronically or by photography while gel is wet. Preserve gel by drying between two cellophane membrane. Hydrate membranes and place gel in between. Remove any air bubbles and allow gel to dry either in a frame or in a gel dryer.

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| 3 |

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