**BIOTECH Project Resource Center Loan Checklist**

Teacher: Date loaned:

School: # groups:

Students/classes: Independent:

**Materials for Protein Analysis of GFP (students work in groups of 4):**

|  |  |  |
| --- | --- | --- |
| Number | Item | Returned? |
| 1 | Microcentrifuge |  |
| 1 | Heat block or hot water bath |  |
| 1 per 2 gels | Vertical polyacrylamide gel electrophoresis box (for mini gels) |  |
| 1 per 2 gels | Power supplies for gel electrophoresis with 150V option |  |
| 1 per gel | White light boxes with adapters |  |
| 1 per two groups | Spectrophotometer with adapter |  |
| 1 per group | UV light |  |
| 1 per group | Sharpie |  |
| 1 | Box Kimwipes |  |
| 1 per group | Microcentrifuge tube rack |  |
| 1 per group | P200 pipette |  |
| 1 per group | Box P200 sterile tips (***please return the box***) |  |
| 1 per group | P1000 pipette |  |
| 1 per group | Box P1000 sterile tips (***please return the box***) |  |
| 3 | 1L bottle of Tris-Glycine-SDS buffer (can be poured back into bottles and reused among classes) |  |
| 1 gel per two groups | Bio-Rad Mini-PROTEAN TGX Precast Gel, 10%, 10-well comb  |  |
| 1 | Mini-PROTEAN Cassette Opening Lever (***for teacher,* *please return)*** |  |
| 1 | Bottle Coomassie blue stain (about 500 ml) – *non-toxic, drain safe; destain gels with water* |  |
| 1 per gel | Plastic tray (to hold gel during staining and destaining) |  |
|  | GFP-transformed *E. coli* with glowing and non-glowing colonies |  |
| 2 per group | 10 µL inoculation loops  |  |
| 2 per group | 1.7 ml tubes with 500 µL LB broth  |  |
| 10 per group | 1.7 mL microcentrifuge tubes |  |
| 1 per group | 1.7 mL tube with 1 mL of Camiolo extraction buffer |  |
| 1 set per two groups | BSA standards 0, 0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 mg/mL (20 µL each) |  |
| 1 per two groups | 24 mL Bradford reagent in amber tube – ***keep refrigerated*** |  |
| 20 per two groups | Cuvettes (***please return unused cuvettes and the foam box***) |  |
| 20 per two groups | Parafilm squares (to use for inverting cuvette to mix sample) |  |
| 1 per group | Cuvette rack |  |
| 1 per group | 500 µL Laemmli buffer |  |
| 1 tube | Protein ladder/molecular weight marker (EZ-Run Pre-Stained Rec Protein Ladder; 5 µL per gel) |  |
| 10 per gel | Gel-loading tips |  |
| 1 | Biohazard bag |  |

**Reagent Descriptions:**

*1X Tris-Glycine-SDS running buffer*

25mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3. Combine 3.03 g Tris base, 14.41 g glycine, and 1 g SDS (wear mask when working with SDS) with 1 liter deionized or distilled water. The pH should be approximately 8.3. Store at room temperature.

*Coomassie blue stain and destain*

Add 0.2 g Coomassie blue to 1 liter of distilled water and mix using a stir bar for 2-3 hours (do not use heat). Add 3 mL of concentrated hydrochloric acid (HCl) for a final concentration of approximately 35 mM (most bottles of concentrated HCl are approximately 12 M). Mix well and store in a sealed, amber bottle at room temperature indefinitely.

Gels can be stained overnight (shaking recommended) in this solution without overstaining the protein bands. Alternatively, gels can be stained until the protein bands are visible enough for analysis (e.g., 30+ minutes). To increase the contrast between the stained protein bands and the rest of the gel, destain the gel in distilled water (shaking recommended) overnight. Gels can be stored in water for several days without losing the protein bands.

*1.0 M Tris-Cl (pH 6.8)*

Combine 60.5 g Tris base with about 350 ml water. Add enough HCl to give the solution a pH of 6.8. Add enough water to make the total volume of the solution 500 ml. Store in a sealed bottle at room temperature indefinitely.

*Laemmli buffer*

To make 100 ml, combine 10 ml 1.0 M Tris-Cl (pH 6.8), 20 ml 20% SDS, 0.1 g bromophenol blue, 20 ml glycerol, and water to make a total volume of 100 ml. This is a 2X recipe, which when mixed with the protein sample for gel loading, will bring it to 1X. Dispense in 0.5 ml aliquots for use by students. Store at room temperature indefinitely

*Camiolo Buffer*

0.075 M potassium acetate, 0.3 M NaCl, 0.1 M L-Arginine, 0.01 M EDTA, 0.25% Triton-100

*Serial dilution BSA standards for Bradford Protein Assay*

1. Collect 7, 50 ml clean (not sterile) centrifuge tubes and label them: 2.0, 1.5, 1.0, 0.75, 0.5, 0.25, 0.125
2. Create 2 mg/ml BSA standard: Add 100 mg BSA to 50 ml centrifuge tube. Bring the volume to 50 ml with nano-pure water. Mix by vortexing until all solids dissolve.
3. Create 1.5 mg/ml BSA standard: Transfer 37.5 ml of 2 mg/ml BSA to a new 50 ml centrifuge tube. Bring the volume to 50 ml with nano-pure water. Mix by inverting.
4. Create 1.0 mg/ml BSA standard: Transfer 33.3 ml of 1.5 mg/ml BSA to a new 50 ml centrifuge tube. Bring the volume to 50 ml with nano-pure water. Mix by inverting.
5. Create 0.75 mg/ml BSA standard: Transfer 37.5 ml of 1.0 mg/ml BSA to a new 50 ml centrifuge tube. Bring volume to 50 ml with nano-pure water. Mix by inverting.
6. Create 0.5 mg/ml BSA standard: Transfer 33.3 ml of 0.75 mg/ml BSA to a new 50 ml centrifuge tube. Bring volume to 50 ml with nano-pure water. Mix by inverting
7. Create 0.25 mg/ml BSA standard: Transfer 25 ml of 0.5 mg/ml BSA to a new 50 ml centrifuge tube. Bring volume to 50 ml with nano-pure water. Mix by inverting.
8. Create 0.125 mg/ml BSA standard: Transfer 25 ml of 0.25 mg/ml BSA to a new 50 ml centrifuge tube Bring volume to 50 ml with nano-pure water. Mix by inverting.
9. Label 1.7 ml micro-centrifuge tubes according to the standard concentration. Aliquot 1.0-1.5 ml of each standard into its corresponding 1.7 ml micro-centrifuge tube.