**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? |
| 8 | microfuge tube racks |  |
| 3 one liter bottles  | plastic bottles of TeoTricine buffer(can be poured back into containers) |  |
| 1 | heat block |  |
| 2 tubes per group | 1.7 ml microfuge tubes |  |
| 4 | Precast SDS-PAGE gels (2 groups per gel)  |  |
| 2 per group | 1.7 ml tubes with 500 μl LB broth  |  |
| 2 per group | 10 μl loops  |  |
| 1 | Centrifuge |  |
| 1 per group | Tube with 1 ml of Camiolo Buffer  |  |
| 8  | UV lights |  |
| 8 | Pipettes (p20 and p1000) |  |
| 5 pipet tip per sample per group | pipette tips (p20 and p1000) |  |
| 2 | Protein gel rigs with wires |  |
| 2 power supplies  | power supplies for 4 rigs with 200V ability |  |
| 4  | light boxes with adapters |  |
| 1 | 1 liter bottle Destain |  |
| 1 | bottle Coomassie blue stain (about 500 ml) |  |
| 4 | plastic trays (to hold gels during staining and destaining) |  |
| 4-8 tubes/grp | Extraction buffer |  |
| 2-4 tubes/grp | Extra extraction buffer for dilution |  |
| 1 per group | BSA standards |  |
| 20 ml/2 groups | Bradford assay |  |
| 20/2 groups | cuvettes |  |
| 2/group | Cuvette rack |  |
| 4 | Specs  |  |
| 4 | Lamelli’s buffer |  |
| 1 | roll masking tape |  |
| 1 | box kimwipes |  |
| 8 | Sharpie pens |  |
| 1 | biohazard bag |  |
| 1  | Protein MW Marker (5 l per gel) |  |
|  | Gel loading tips |  |
|  |  |  |
|  |  |  |

When you are reloading the bins, please check off each item in the 'Returned?' column as a double-check that all those little pieces of equipment get packed. Thanks!

If you have questions about experiments or materials, please feel free to contact the BIOTECH Project at:

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**ProteinAnalysisGFP\_TeacherGuide.docx**

**Recipes**

*5X stock of Tris-Glycine-SDS buffer*

Combine 15.1 g Tris base, 94 g glycine, and 50 ml 10% SDS (5 g SDS with 45 ml water) with water to make a total volume of 1 liter. Dilute this 1:4 with water (i.e. 100 ml stock with 400 ml water to make a total 500 ml of Tris-Glycine-SDS buffer or 200 ml stock with 800 ml water to make 1 Liter). Store in a sealed bottle at room temperature indefinitely.

*Coomassie blue stain*

For each liter of stain, combine 450 ml water, 2.5 g Coomassie blue, 450 ml methanol, and 100 ml glacial acetic. Store in a sealed bottle at room temperature indefinitely.

*Destain*

For each liter of destain, combine 600 ml water, 300 ml methanol, and 100 ml glacial acetic acid. Store in a sealed bottle at room temperature indefinitely.

*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 (0.5 liter). Store in a sealed bottle at room temperature indefinitely.

*Sample buffer*

To make 100 ml sample buffer, 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 actually a 2X recipe, but the sample buffer is used at 2X, not diluted. Dispense in 0.5 ml aliquots for use by the 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*

The following steps can make use of a used, clean but not sterile, 50ml centrifuge tube

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