**BIOTECH Project Resource Center Loan Checklist**

Teacher: Date loaned:

School: # groups:

Students/classes: Independent:

**Materials for protein fingerprinting using agarose gel lab (students work in groups of 4)**:

|  |  |  |
| --- | --- | --- |
| Number | Item | Returned? |
| 1 | Hot water bath with lid |  |
| 1 | Heat block |  |
| 2 | Thermometer in plastic holder |  |
| 3 liters | Plastic bottles of Tris-glycine-SDS buffer (can be poured back into containers and reused) |  |
| 1-2 bottles per class | Glass bottles of agarose (200 ml 3% agarose in Tris-glycine buffer in 500 ml bottle) |  |
| 8 | Blue pipettes (50 µL) or P200 micropipettes |  |
| 12/group | 20-200 µL tips |  |
| 8 | Gel electrophoresis boxes with 2 stoppers and 1 comb each |  |
| 8 | Pairs of red and black wires |  |
| 4 dual or 8 individual power supplies | Power supplies for gel electrophoresis |  |
| 8 | White light boxes with adapters |  |
| 2 | Bottle Coomassie blue stain (about 500 ml) – *non-toxic, drain safe; destain gels with water* |  |
| 8 | Microcentrifuge tube racks |  |
| 4 per group | 1.7 mL microcentrifuge tubes |  |
| 4 per group | 1.7 mL tubes with 500 µL sample (i.e., Laemmli) buffer |  |
| 1 per group | Plastic trays or weigh boats to hold gels during staining/destaining |  |
| 4 tissues per group | Tissues for protein extraction |  |
| 1 | Biohazard bag |  |

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:

Daryn Stover

dastover@arizona.edu

(520) 626-4664

**Have fun!**

**Reagent Descriptions:**

*1X Tris-glycine buffer*

Combine 3.02 g Tris base and 18.8 g glycine with water to make a total volume of 1 liter. Use for making 3% agarose.

*3% agarose in Tris-glycine buffer*

In a 500 ml Pyrex bottle, combine 6 g agarose with 200 mL **Tris-glycine buffer** (do NOT use Tris-glycine SDS buffer). Microwave uncovered for 1 minute at a time until agarose is dissolved, taking care not to let the agarose boil over on the microwave or your hand. Cool briefly before pouring in gel caster, or cover loosely and store at room temperature to remelt and use later.

*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 (e.g., 100 mL stock with 400 mL water). Store in a sealed bottle at room temperature indefinitely.

*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 distilled water for a total volume of 350 mL. Add enough HCl to give the solution a pH of 6.8. Bring the final volume of the solution up to 500 mL with distilled water. Store in a sealed bottle at room temperature indefinitely.

*Sample buffer (i.e., 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 distilled 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.