CHEM525 Experiment 5
Buffer and Gel Preparation

Purpose and Theory:

Over the next two months, we'll be purifying LDH from *E. coli* cells and then spending several weeks examining its activity under a variety of experimental conditions. The goal of lab this week is to prepare all buffers, gels, and solutions that we will need.

Safety Precautions

- Always wear glove, safety coat and goggles when in the lab.
- If a significant amount of any chemical is spilled, immediately seek the instructor for clean-up protocols.
- Acrylamide is a neurotoxin. Handle it with care. Once polymerized, it is no longer dangerous.

Procedure

1. Make each of the following:
    a. 1 L of 50 mM Tris Buffer at pH 8.0 with 50 mM NaCl.
    b. 250 mL of 200 mM Imidazole in the buffer made in part a.
    c. 250 mL of 1 M NaCl in 50 mM Tris Buffer at pH 8.0.
    d. 500 mL of 20 mM Tris Buffer at pH 8.0 with 50 mM NaCl.
    e. 250 mL of 20 mM Tris Buffer at pH 8.5 with 50 mM NaCl.
    f. 250 mL of Bis-Tris Buffer at pH 6.0 with 50 mM NaCl.
    g. 250 mM of Bis-Tris Buffer at pH 7.0 with 50 mM NaCl.

2. Make 1 L of Lauria Bertani Broth (25 g/L)

3. Make 1 15% SDS-PAGE Gel according to instructor directions.