Ubiquitin E2 Charging Assay

1. Setup thioester reactions in 1.5 mL tubes as follows:
    a. 4 µL of E1 (0.5 µg/µL) or 4 µL dH_2O as a control
    b. 2 µL His-UbcH2 (1 µg/µL) or 2 µL UbcH2 ΔTail
    c. 2 µL 10X thioester buffer
    d. 6.5 µL Flag-Ubiquitin (1 µg/µL)
    e. 5.5 µL dH_2O
2. Incubate at room temperature for 10 min
3. Add 20 µL 2X non-reducing SDS sample buffer (homemade)
4. For reduced sample:
    a. Remove 15 µL into a new tube
    b. Add 0.8 µL of 1 M DTT (reduced sample only)
    c. Boil for 5 min (reduced sample only)
5. For Non-reduced sample:
    a. Incubate 15 min at 30 °C
6. Run 15 µL on SDS-PAGE gels. *Run at high voltage to prevent spontaneous reduction. Do not run reducing and non-reducing samples near each other, at least several lanes apart or on different gels.*
7. Coomassie stain gel

<u>10X Thioester buffer</u>
100 mM HEPES pH 7.5
1 M NaCl
400 µM ATP
20 mM $MgCl_2$