COOMASSIE BLUE STAIN

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Preparation:

1. Remove 1.5M Tris (pH 8.8) (w/ SDS) from 4°C fridge
2. Remove 1.0M Tris (pH 6.8) (w/ SDS) from 4°C fridge
3. Remove 30% acrylamide mix from 4°C fridge
4. TEMED
5. dH_2O
6. 70% ethanol
7. Coomassie dye recipe (the order of preparation is critical):
    a. Dissolve 25g of Aluminum Sulfate in 200 mL of Millipore water to create a 5% w/v concentration
    b. Add 50 mL of 100% ethanol for a final concentration of 10% v/v
    c. Dissolve 0.1 g of Coomassie Brilliant Blue G-250 (Sigma) to create a 0.02% w/v concentration; immediately mix well by swirling and inverting the bottle repeatedly
    d. Add 12 mL of concentrated o-phosphoric acid for a final concentration of 2% v/v. The addition of the acid to the ethanol will facilitate colloid production.
    e. Fill up to 500 mL with Millipore water

Protocol

1. Assemble gel cassette
    a. Place gray rubber strip on the bottom of one of the two 'clip on' stations (large plastic 'stations')
    b. Take two glass plates (one has to be 1.0mm plate) and wash them gently with soap water
        i. Wear gloves
        ii. Hold plate by the edges
        iii. Allow to air dry on a paper towel
    c. Place the two plates together and insert them into the green holder
        i. Secure by snapping the sides
        ii. Ensure the bottom is even to prevent leaking
    d. 'clip' the 1.0mm glass plate/green holder unit onto the plastic station
    e. Add 5 mL of water to test for leakage
        i. Dump water
        ii. Dry gently with Kim wipe
2. Prepare Separating Gel (make 1.5x the amount to be safe)
    a. 1.5 mL dH_2O
    b. 2.3 mL 30% acrylamide

c. 1.3 mL 1.5M Tris (pH 8.8) (w/ SDS)
d. 40 µl 10% APS
    i. Weigh 0.1 g of APS powder and dissolved in 1 mL of dH_2O
    ii. Shake solution gently
e. 2 µl TEMED
    i. Shake solution

3. Add gel to cassette
    a. Add ~5 mL of Separating Gel to cassette
    b. Draw up remaining in a 1 mL pipet
    c. Wait 20 minutes (could take longer) for polymerization to occur
        i. Test by regularly ejecting pipet to see if gel has polymerized
    d. Add 70% ethanol to cassette (up to the brim of the two glass plates)

4. After polymerization has occurred, pour out ethanol and wash with dH_2O. Dry with Kim wipe (gently) as best as possible

5. Prepare Stacking Gel (make 1.5x amount to be safe)
    a. 863 µl H_2O
    b. 213 µl 30% acrylamide mix
    c. 156 µl 1.0M Tris (pH 6.8) (w/ SDS)
    d. 13 µl 10% APS
        i. Shake
    e. 1 µl TEMED
        i. Shake

6. Add Stacking Gel to cassette
    a. Add enough gel to reach brim of glass plates
        i. Takes ~3 mL
    b. Insert green comb straight into gel
    c. *turning on heat block now will save time – turn temperature to 95°C*
    d. Gel will polymerize quickly – wait 5 min. (could take longer)
    e. Once gel has polymerized, remove comb by lifting **vertically** and gently

7. Prepare samples
    a. Prepare a DTT + 3x sample buffer solution
        i. Add 250 µl DTT (found in -20°C freezer in box labelled 'common reagents') **per** 750 µl 3x SDS sample buffer (dark purple solution)
    b. Add 50 µl of DTT solution **per** 100 µl of cell sample
    c. Place sample on 95°C heat block for 10 minutes

8. Loading Samples
    a. Make 1x running buffer
        i. Add 240 mL of 5x stock solution to 960 mL MilliQ water for total volume of 1.2 L
    b. Remove two glass plates with gel from green holder

c. Insert glass plates into plastic holder that can hold two sets of plates
    i. Pull apart the side locking handles
    ii. Place glass plate into one of the sides (1.0 mm plate facing inward)
    iii. Place a purple plastic plate on the other side (words facing inwards)
    iv. Lock in place
d. Place plastic holder in tank with corresponding currents correct
e. Add 20 µl of protein ladder (found in -20^{o}C fridge in common reagents box) to first well
f. Add 30 µl of each sample into subsequent wells

9. Running Samples
    a. Pour 1.2L of 1x running buffer into tank
    b. Push bubbles away from the plastic holder if there are any
    c. Place lid on with correct currents
    d. Run sample for ~40 minutes at 200V
        i. Check to see if small bubbles rise from bottom of the tank

10. Remove gel gently and place onto large Petri dish
    a. Wash twice with Millipore water to remove excess SDS
    b. Submerge with required stain and place on shaker overnight
    c. Bands should appear within 2 hours but overnight exposure will maximize the appearance of bands; the gel will have appeared to have shrunken but this is normal

11. Destain the gel by washing repeatedly with Millipore water