Date: _____

Immunoblot to detect reactivity of CF sera to nBPI, Ct-BPI, and Ct-BPI recombinant proteins

1. Prepare samples
    a. Make up loading buffer (LB): [2X stock solution: 100ul 1M Tris pH6.8, 100ul Bromophenol Blue, 200ul 1M DTT, 400ul 10% SDS, 200ul glycerol]
        i. Dilute to 1X in dH2O
    b. Prepare labeled 1eppendorf tubes → and sample and loading buffer (30ul total): e.g. if sample 2ul add 2 ul 2X LB and 26ul 1X loading buffer
    c. Boil the samples for 3' and keep at RT until use

Sample	Amount to load (ug)	Volume protein (ul)	Volume LB 2X (ul)	Volume LB 1X (ul)
nBPI (100ug/ml)	1.2			
Ct-BPI (50 ug/ml)	1.2			
BPI1m (68.2 ug/ml)	1.2			
BPI2m (46.5 ug/ml)	1.2			
BPI3m (47.4 ug/ml)	1.2			

1. Assemble 12% SDS-PAGE gels (Bio-Rad, 4561043)
2. Running Buffer (5X) – 1L → Dilute to 1X before running SDS-PAGE
    a. 15g Tris
    b. 72g glycine
    c. 5g SDS

3. Load the samples (25ul each well) and run the SDS-PAGE gel at 100V until runs off (~1.5hr)
    a. For ladder, dilute 1:1 (from stock) with 2X LB (also 25ul per well)

b) Immunoblot with CF serum and 2' HRP-labeled ab

4. Prepare 10X Transfer Buffer – 1L
    a. 30.3g Tris
    b. 144g glycine
5. Prepare transfer buffer using 10X stock:
    a. 100ml 10X stock
    b. 200ml methanol
    c. 700ml H2O

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6. Assemble the sandwich cassettes and transfer samples onto nitrocellulose membrane
    a. Cut out blotting paper and nitrocellulose membrane → Soak in transfer buffer
    b. Remove gels from the glass plates, cut off the stacker
    c. Set up the transfer apparatus: black side on the bottom → sponge → blot paper → gel → nitrocellulose membrane → blot paper → sponge → white side
    d. Insert cassette black to red, put an ice pack (and a small stir bar under ice pack) and fill with transfer buffer
    e. Transfer at 100V for 60min while stirring

7. Block nitrocellulose membrane in **TBS-Tween (TBS-T) + 10% FCS** o/n at R.T.

8. Prepare 2L of TBST in dH2O
    a. 20mM Tris/ pH 7.5
    b. 150mM NaCl
    c. 0.1% Tween 20

9. Wash with TBS-T + 3% FCS (Make about 1L)
    a. 1 X 15min
    b. 3 X 5min

10. Incubate with 10ml CF serum (1:100 in TBST+3% FCS) on the shaker for 3hrs at RT

11. Wash with TBS-T + 3% FCS
    a. 1 X 15min
    b. 3 X 5min

12. Incubate with 10ml goat anti-mouse HRP labeled 2' ab (1:3,000 in TBS-T + 3% FCS) for 1hr at RT on the shaker

13. Wash 3X with TBS-T + 3% FCS
    a. 1 X 15min
    b. 5 X 5min

14. Add West Pico substrate and expose the membrane
    a. For West Pico, uses 1mL for a full-sized blot. If using half-sized blot, scale down to 500uL. Pipette directly onto the blot and tilt until solution has fully covered the blot.