Western Blot

Transfer:
Soak 1 whatman paper in anode buffer 1 (buffer 1), 2 whatman papers in anode buffer 2 (buffer 2), and 3 whatman papers in cathode buffer (buffer 3)
Soak the nitrocellulose membrane in ddH_2O
The transfer order is as follows:

Positive
1 whatman paper (Buffer 1)
2 whatman papers (Buffer 2)
Nitrocellulose (.45 um)
Gel
3 whatman papers (Buffer 3)
Negative

Transfer at 500 milliamps for 1 hour.

Probing:
Prepare 500 mL 1x PBST (.1-1% Tween 20)
Cut blot at the desired locations
Block all pieces in 200 mL 1x PBST + 10% milk for 30 minutes
Dilute the primary antibodies in 1x PBST + 5% milk (1:1000 in 50 mL)
Incubate each piece of the western in the appropriate antibody for 2 hrs (or overnight) on the rocker
Wash the blots together in 1x PBST 3 times for 5 minutes each
Dilute the secondary antibodies in 1x PBST + 2.5% milk (1:20000 in 50 mL)
Incubate the blots in the appropriate secondary antibody for 1-2 hours on the rocker
Wash the blots together with 1x PBST 3 times for 5 minutes each
Put the blot back together on a piece of saran wrap
Mix the ECL reagents (1 mL of each) and pipette evenly over the blot
Allow for an incubation period of a minute or so
Take a 1 minute exposure and then based on this first exposure take a 5 or a 16 minute