

# NOTEBOOK-READY PROCEDURE

## Western Blot Procedure

**Experiment/Project #:** \_\_\_\_\_

**Name(s):** \_\_\_\_\_

**Date(s):** \_\_\_\_\_

Material	Company/Catalog #/Lot #
Primary Antibody:	
Secondary Antibody:	
Tertiary Antibody:	
Wash Solution:	
Blocker:	
Substrate:	
Protein Ladder:	
Equipment	Company/Catalog #/Lot #
Imager/Film Processor:	
Camera:	

**1. MEMBRANE TYPE**

Nitrocellulose       PVDF       Nylon

**2. LANE IDENTIFICATION**

Lane 1: \_\_\_\_\_ Lane 5: \_\_\_\_\_ Lane 9: \_\_\_\_\_  
 Lane 2: \_\_\_\_\_ Lane 6: \_\_\_\_\_ Lane 10: \_\_\_\_\_  
 Lane 3: \_\_\_\_\_ Lane 7: \_\_\_\_\_ Lane 11: \_\_\_\_\_  
 Lane 4: \_\_\_\_\_ Lane 8: \_\_\_\_\_ Lane 12: \_\_\_\_\_

**3. BLOCK MEMBRANE**

1 hour at RT or overnight at 4° C; Blocker: \_\_\_\_\_ Temperature: \_\_\_\_\_  
 Actual time: Start - \_\_\_\_\_ AM/PM      End - \_\_\_\_\_ AM/PM      Total - \_\_\_\_\_

**4. PRIMARY ANTIBODY INCUBATION**

30 – 60 minutes at RT, or overnight 4° C; Temperature: \_\_\_\_\_  
 Actual time: Start - \_\_\_\_\_ AM/PM      End - \_\_\_\_\_ AM/PM      Total - \_\_\_\_\_  
 Antibody: \_\_\_\_\_  Polyclonal  Monoclonal  
 Antibody diluted in blocker: \_\_\_\_\_  
 Conjugate:  N/A  HRP  AP  Other Tag: \_\_\_\_\_  
 Purity:  Purified  Unpurified  Serum  
 Antibody Concentration: \_\_\_\_\_ (µg/mL) or Dilution: \_\_\_\_\_

**5. WASH MEMBRANE**

3 x 5 minutes  
 Wash solution: \_\_\_\_\_  
 Actual time: Wash 1 - \_\_\_\_\_ Wash 2 - \_\_\_\_\_ Wash 3 - \_\_\_\_\_

**6. SECONDARY ANTIBODY INCUBATION  N/A**

30 – 60 minutes at RT, or overnight 4° C; Temperature: \_\_\_\_\_  
 Actual time: Start - \_\_\_\_\_ AM/PM      End - \_\_\_\_\_ AM/PM      Total - \_\_\_\_\_  
 Antibody: \_\_\_\_\_  Polyclonal  Monoclonal  
 Antibody diluted in blocker: \_\_\_\_\_  
 Conjugate:  N/A  HRP  AP  Other Tag: \_\_\_\_\_  
 Purity:  Purified  Unpurified  Serum  
 Antibody Concentration: \_\_\_\_\_ (µg/mL) or Dilution: \_\_\_\_\_

- 7. WASH MEMBRANE**  N/A  
 3 x 5 minutes  
 Wash solution: \_\_\_\_\_  
 Actual time: Wash 1 - \_\_\_\_\_ Wash 2 - \_\_\_\_\_ Wash 3 - \_\_\_\_\_
- 8. TERTIARY ANTIBODY INCUBATION**  N/A  
 30 – 60 minutes at RT, or overnight 4° C; Temperature: \_\_\_\_\_  
 Actual time: Start - \_\_\_\_\_ AM/PM End - \_\_\_\_\_ AM/PM Total - \_\_\_\_\_  
 Antibody: \_\_\_\_\_  Polyclonal  Monoclonal  
 Antibody diluted in blocker: \_\_\_\_\_  
 Conjugate:  N/A  HRP  AP  Other Tag: \_\_\_\_\_  
 Purity:  Purified  Unpurified  Serum  
 Antibody Concentration: \_\_\_\_\_ (µg/mL) or Dilution: \_\_\_\_\_
- 9. WASH MEMBRANE**  N/A  
 3 x 5 minutes, 1 x 15 minutes  
 Wash solution: \_\_\_\_\_  
 Actual time: Wash 1 - \_\_\_\_\_ Wash 2 - \_\_\_\_\_ Wash 3 - \_\_\_\_\_ Wash 4 - \_\_\_\_\_
- 10. SUBSTRATE INCUBATION**  
 Final washes:  
 1 x 5 minutes wash Actual time: \_\_\_\_\_  N/A  
 1 x 5 minutes Reagent Quality water Actual time: \_\_\_\_\_  N/A  
 Substrate Name: \_\_\_\_\_  Colorimetric  Chemiluminescent  Fluorescent  
 Recommend development time: \_\_\_\_\_ minutes  
 Actual time: \_\_\_\_\_ minutes  
 Blotted dry  Left membrane wet  Liquid pooled on top
- 11. MEMBRANE DEVELOPMENT**  
 Film  Imager  Other  
 Development time: \_\_\_\_\_ minutes  
 Computer file name: \_\_\_\_\_ Location: \_\_\_\_\_  N/A
- 12. RESULTS/CONCLUSIONS**

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