

Experimental Accuracy Western Blot Assay

Experiment _____ Scientist _____ Date _____

Sample Preparation

Protein Loading Buffer Mfg & Cat # _____ Lot # _____ Expiration _____

BME/DTT Mfg & Cat # _____ Lot # _____ Expiration _____

MW Markers Mfg & Cat # _____ Lot # _____ Expiration _____

Heat Samples _____ min @ _____ °C

1X Running Buffer: _____ mL _____ Buffer + _____ mL water/gel unit.

_____ Buffer Mfg & Cat # _____ Lot # _____ Expiration _____

Antioxidant Mfg & Cat # _____ Lot # _____ Expiration _____

Gel Format: _____ % Mfg & Cat # _____ Lot # _____ Expiration _____

Loading Order:

Gel #1 of _____ gels

Experimental Design:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10. _____
11. _____
12. _____
13. _____
14. _____
15. _____

Run @ _____ for _____ minutes

[] Transfer _____

[] Nitrocellulose [] PVDF Mfg & Cat # _____ Lot # _____ Expiration _____

[] Stain _____

Notes: _____

Western Blot:

Date: _____

Materials: Blocking Buffer:

Mfg & Cat # _____ Lot # _____ Expiration _____

Blocking Buffer + 0.2% Tween® 20: _____ added 1:100 of 20% Tween 20 solution

Primary Antibody(ies):

Mfg & Cat # _____ Lot # _____ Expiration _____

Mfg & Cat # _____ Lot # _____ Expiration _____

Mfg & Cat # _____ Lot # _____ Expiration _____

Secondary Antibody(ies):

Mfg & Cat # _____ Lot # _____ Expiration _____

Mfg & Cat # _____ Lot # _____ Expiration _____

Chemi Substrate:

Mfg & Cat # _____ Lot # _____ Expiration _____

10X PBS/TBS:

Mfg & Cat # _____ Lot # _____ Expiration _____

10X Tris Glycine:

Mfg & Cat # _____ Lot # _____ Expiration _____

Tween 20:

Mfg & Cat # _____ Lot # _____ Expiration _____

Method:

- ___ 1. Dry Membrane after transfer. Rehydrate nitrocellulose membrane for ~ 5 minutes in PBS/TBS or if using PVDF membrane, briefly in methanol, then PBS/TBS for 5 minutes.
- ___ 2. Incubate 1 hour at room temperature in blocker (no detergent) with gentle agitation.
On @ _____
Off @ _____
- ___ 3. Decant block and add primary antibody diluted 1: _____ in blocker + 0.2% Tween® 20. Incubate 1 hour at room temperature with gentle agitation.
On @ _____
Off @ _____
- ___ 4. Decant and wash 4X, 5 minutes each wash, with agitation, using 1X PBS/TBS + 0.1% Tween 20
 Wash step 1 Wash step 2 Wash step 3 Wash step 4
- ___ 5. Decant final wash and add secondary antibody diluted 1: _____ in blocker + 0.2% Tween 20. Incubate 1 hour at room temperature in the dark with gentle agitation.
On @ _____
Off @ _____
- ___ 6. Decant and wash 4X, 5 minutes each wash, with agitation, using 1X PBS/TBS + 0.1% Tween 20
 Wash step 1 Wash step 2 Wash step 3 Wash step 4
- ___ 7. Final wash in 1X PBS/TBS only, 5 minutes at room temperature with gentle agitation.
 Final wash step
- ___ 8. Add substrate: _____ mL for _____ minutes
- ___ 9. Image on _____ Parameters: _____

Notes: _____

