

## Western Blot Quiz Questions And Answers

Name (last, first) \_\_\_\_\_

BIO 347 2019 Quiz #5

1. What reagent is used for the Bradford assay (please don't say Bradford reagent!) (2 points)

Coomassie brilliant blue

2. What is the purpose/function of the SDS in SDS/PAGE? Give 2 functions for full credit. (4 points)

it unfolds the proteins, and coats them to give a more uniform charge: mass ratio

3. What is the purpose/function of beta mercaptoethanol or dithiothreitol in SDS-PAGE? (1 point)

it reduces/breaks disulfide bonds

4. Why are the proteins transferred from the gel to a nitrocellulose membrane during Western blotting? (1 point)

because the gel is very fragile, while the membrane is sturdier and withstands the later washing steps better than the gel would

5. What antigen did we try to detect in our Western blot experiment? (2 points)

beta tubulin

6. Why was the preblocking step included in our Western blots (i.e. what would have happened if we had omitted that step)? (2 point)

it coats the membrane, forcing the primary antibody to compete for its specific binding sites against the nonspecific coating of the preblock proteins

7. What is the purpose of the secondary antibody in the Western blot? (1 point)

to bring in an enzyme whose reaction product will be visible/will make the location of primary antibody binding visible

8. What enzyme was conjugated to the secondary antibody in our Western blots? (2 points)

alkaline phosphatase

9. Why did we include the Kaleidoscope marker proteins on your gels? (2 points)

to allow comparisons of migration of our unknown size beta tubulin bands versus the migration of the known Kaleidoscope molecular weight marker proteins

10. What information can Western blotting give that Mancini assays and ELISA assays cannot? (1 point)

### Western blotting can give molecular weight of the protein

12. What was the general relationship between molecular weight and migration of a protein? (2 point)

the larger the protein, the more slowly it migrated down the gel/away from the loading wells