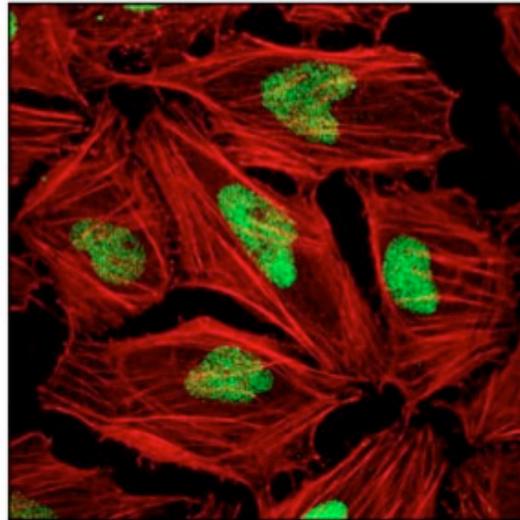


**Guide Questions for Topic: Antibodies**

1. What type of molecule are antibodies?
2. What role do antibodies play within higher eukaryotes (e.g., mammals)?
3. What does the term “antigen” refer to?
4. What is the difference between the “constant region” and “variable region” of an antibody?
5. If I handed you a 1.5 mL tube and told you it contained anti-PUMA antibody, which of the following describes the contents of the tube?
6. You are interested in acquiring an antibody that will bind to the protein MDM2. To do so, you generate synthetic MDM2 protein, inject it into a laboratory rabbit, and allow several days for the rabbit’s immune system to accumulate anti-MDM2 antibodies. After that period of time, what final step must you conduct before you can use the antibody in an experiment?
7. If told you that the anti-PUMA antibody mentioned in question 5 is “conjugated to a fluorophore”, what would that mean?
8. If you wanted to examine where in cells the protein NAB2 localizes, which of the following experiments/techniques would best help you determine this?
9. What does the term “cell lysate” refer to?
10. Which of the following describe the four fundamental steps of Western blot (assuming you are starting with a cell lysate)?
11. If you wanted to isolate endogenous ATM protein from cells, which technique would use to do so?
12. Recall that step 3 of the immunoprecipitation (IP) procedure (as outlined in the lecture slides) entails adding “beads” to the (IP) sample. What two properties of the beads are critical to the success in the IP?
13. Recall from the Topic: Translational Regulation that the protein 4E-BP inhibits global translation through protein-protein interactions with eIF4E when cells are in the absence of growth factors. If you wanted to confirm that 4E-BP and eIF4E form protein-protein interactions, you could grow cells in culture, deprive them of growth factors, and then conduct \_\_\_\_\_.

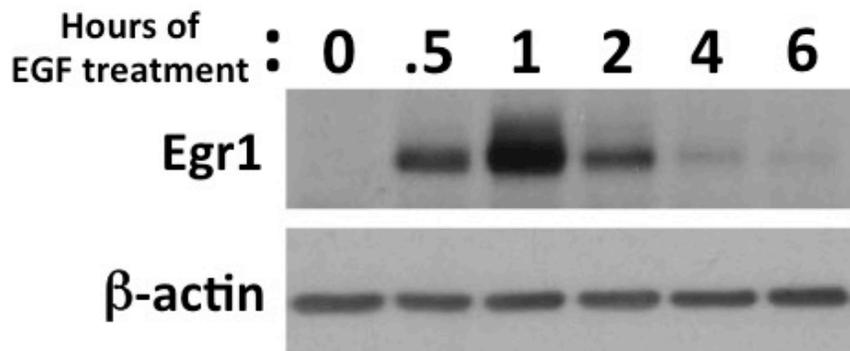
(Questions 14 and 15 are on the next page.)

14. You are a cell biologist interested in determining where the protein MCM2 localizes in cells. To do so, you grow human cells in culture and subject them to immunocytochemistry with two antibodies: (1) anti-MCM2 antibody conjugated to a green fluorophore and (2) anti-actin antibody conjugated to a red fluorophore. A fluorescence micrograph of your data is shown below:



Based on your data, where does MCM2 localize in cells?

15. You are interested in examining how the growth factor EGF affects levels of the transcription factor Egr1. To do so, you grow cells in culture, treat them with EGF for 0, 0.5, 1, 2, 4, or 6 hours, and then create cell lysates from each culture. You then subject the lysates to Western blot analysis for two proteins: Egr1 and  $\beta$ -actin. Your data is shown below:



Based on your data, how does EGF affect Egr1 levels?