Biochemistry Lab

Protein Interaction Design

Study Guide

Now that you've been studying the background for how protein-protein interactions take place and why these are important you will use this guide to plan.

Review the links about the protein interactions to learn the many ways protein-protein interactions are determined. Then read the information on Pull-Down assays – your groups will be developing these assays to measure protein-protein interactions between MDH and CS. Then read through the specific His-tagged pull-down protocols. Finally, together with your group – discuss and answer each of the following questions:

1) Discuss the physical nature of several techniques used to measure protein-protein interactions. Describe the approach used by these techniques and compare/contrast the approach and biochemical nature of the interactions. Include the pull-down method in this work

2) Investigate and report the difference between pull-down and co-immuno precipitation for determining protein interactions.

3) If you don't remember, review what an affinity His tag is and how in the tag, six or more histidine residues bind to nickel or other metals . Review how these tags work in purification and how the His affinity tag is used to bind protein to a "bead". You may want to ask what a chromatography bead is…

4) What is a TEV protease and what is the protease site? In the very first introduction to MDH-CS interactions, your instructor presented that MDH and CS both have a protease site between the His tag and the rest of the protein on the C-terminus. Thinking of the pull-down assay, why is the inclusion of the TEV site important?

5) Sketch out a simple experimental workflow for a pull-down starting with purified MDH and CS.

6) What controls do you need to think about when designing a pull-down assay?

7) There are several formats for pull-down assay including spin columns, traditional beads in a microfuge tube and magnetic beads. What are the differences in how they are used for the assay?

8) Thinking back to the introduction to protein-protein interactions, are there buffer or other components you may want to consider when planning a pull-down assay?