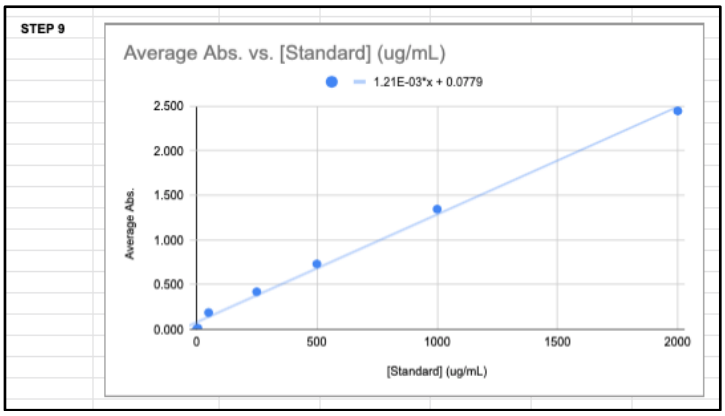
Protocol Discussion 1 Worksheet Solutions

BCA Assay: Absorbance Analysis



STEP 11	Sample Type	Average Abs.	[Sample] (ug/mL)
	PC9	0.1535	62.47933884
	PC9 AXL KO	0.1865	89.75206612

Your protein concentration for PC9 should be in cell D107 and your protein concentration for PC9 AXL KO should be in cell D108. Which one has a higher protein concentration? Why do you think it has a higher protein concentration?

PC9 AXL KO has the higher protein concentration. In order to remove the PC9 gene, the ubiquitination machinery was introduced to the cell, increasing the amount of protein present.

Assignment

- 72 hours to complete

1. From a stock solution of 2 M KCl, you need to prepare 50 mL of 600 mM KCl. How much water and KCl do you need to add? Please include all the steps of the calculation in your response.

   Dilution of 2M KCl Stock Solution to 50 mL of 600 mM KCl Solution

   To determine the **volume of stock KCl** that we need to add, utilize the equation:
   $M_1V_1 = M_2V_2$
   and solve for V_1
   $V_1 = \frac{M_2V_2}{M_1}$

   In this equation:
   M_1= molarity of KCl stock solution
   V_1= volume of KCl stock solution used to prepare the new KCl solution = V_{KCl}
   M_2= molarity of the new KCl solution
   V_2= final volume of the new KCl solution

   Given the values in this problem:
   M_1= 2 M
   M_2= 600 mM $\cdot \frac{1\ M}{1000\ mM}$ = 0.6 M
   V_2= 50 mL

   Substitute and solve for $V_1 = V_{KCl}$:
   $V_1 = \frac{M_2V_2}{M_1}$
   $V_1 = \frac{(0.6\ M)(50\ mL)}{(2\ M)}$
   $\boxed{V_1 = 15\ \text{mL} = V_{KCl}}$

   To determine the **volume of water** that we need to add, utilize the equation:
   $V_{Total} = V_{KCl} + V_{Water}$
   and solve for V_{Water}
   $V_{Water} = V_{Total} - V_{KCl}$

   In this equation:
   V_{Total} = total volume of new solution
   V_{KCl} = volume of KCl stock solution used to prepare the new KCl solution
   V_{Water} = volume of water used to dilute the new KCl solution to the desired concentration

   Given the values in this problem:
   V_{Total} = 50 mL
   V_{KCl} = 15 mL

   Substitute and solve for V_{Water}
   $V_{Water} = V_{Total} - V_{KCl}$
   V_{Water} = 50 mL – 15 mL
   $\boxed{V_{Water} = 35\ \text{mL}}$

   To make the new solution, 15 mL of stock KCl solution and 35 mL of water must be added.

2. Below is the information for the KCl, water and 50 mL conical tube. Please write a protocol on how to prepare the 50 mL of 600 mM KCl solution in a 50 mL conical tube. The protocol should be clear and detailed so a person can complete this task with minimal supervision. Include all the steps and all the tools and consumables needed.
    - 2M KCl: https://www.thermofisher.com/order/catalog/product/AM9640G?us&en#/AM9640G?us&en
    - Water: https://www.thermofisher.com/order/catalog/product/10977015#/10977015
    - 50 mL conical tube: https://www.thermofisher.com/order/catalog/product/339650?SID=srch-srp-339650#/339650?SID=srch-srp-339650

    Preparation of 50 mL of 600 mM KCl Solution from 2M KCl Stock Solution

    Additional Materials Required:

    - Paper Towels
    - 70% Ethanol Solution: https://www.fishersci.com/shop/products/denatured-ethanol-solution-70-molecular-biology-grade-fisher-bioreagentstrademark-tm/BP82031GAL
    - 2 50 mL Serological Pipettes: https://www.thermofisher.com/order/catalog/product/170376N#/170376N
    - 1 Motorized Pipette Filler (that is compatible with 50mL pipettes): https://www.labdepotinc.com/p-59649-levo-plus-motorized-pipette-filler?utm_source=google&utm_medium=cpc&utm_campaign=smart-shopping&gclid=Cj0KCQiA6Or_BRC_ARIsAPzuer_ef82Bkq33Mz3wv9xwHwJabkVteBS0DZldWuHGSL4iC-Z4HTo9ZJIaAlc4EALw_wcB
    - 50 mL Conical Tube Rack: https://www.fishersci.com/shop/products/globe-scientific-centrifuge-tube-racks/21200285
    - Vortex Mixer: https://www.scientificindustries.com/vortex-genie-2.html?gclid=Cj0KCQiA6Or_BRC_ARIsAPzuer9jImPvAa1d-TEAOW1Tm1UGCxj6YMfBZOKg_fMLbbMgopdQn3Th3iYaAt6HEALw_wcB

1. Put on Personal Protective Equipment (Latex Gloves, Lab Coat, Googles).
2. Clean the work station.
    - Remove unnecessary materials from the work station.
    - Use paper towels to wipe the lab bench down with 70% ethanol solution.
3. Remove the water, 2M KCl, and conical tube from room temperature storage and place them onto the lab bench.
4. Obtain two, individually wrapped 50 mL serological pipettes and the motorized pipette filler. Place the materials on the lab bench and plug the motorized pipette filler into a nearby outlet.
5. Open one of the 50 mL serological pipettes. While keeping the serological pipette in its wrapping, attach the top of the serological pipette to the motorized pipette filler.
    - Keeping the pipette in its wrapping while it is not being used reduces the risk of contamination and allows for easier cleanup at the end of the preparation process.
6. Open the water container and place the lid top-side-down onto the lab bench.
    - The lid is placed top-side down so that the inside of the water lid is not in direct contact with the bench top.
7. Take the 50 mL conical tube in your non-dominant hand and twist off the cap, holding the lid to the side of the tube. Take the motorized pipette filler in your dominant hand and slide the pipette out of its packaging as you lift up the filler.
8. Place the pipette in the water container and draw 35 mL of water into the serological pipette.
    - While performing this step, withdraw the water slowly and keep the pipette tip under the surface of the water to ensure that there are no air bubbles.
9. Transfer the 35 mL of water into the conical tube.
10. Place the 50 mL pipette back into its individual wrapping and detach the pipette tip from the filler. Close the conical tube and place the conical tube in the tube rack.
11. Close the water container.
12. Open the other 50 mL serological pipette. While keeping the serological pipette in its wrapping, attach the top of the serological pipette to the motorized pipette filler.
13. Open the KCl container and place the lid top-side-down onto the lab bench.
14. Repeat Step 7.
15. Place the pipette in the 2M KCl container and draw 15 mL of water into the serological pipette.
    - While performing this step, withdraw the KCl slowly and keep the pipette tip under the surface of the water to ensure that there are no air bubbles.
16. Transfer the 15 mL of KCl into the conical tube. After transferring the KCl, mix the solution by slowly drawing and withdrawing liquid with the pipette.
17. Repeat Step 10.
18. Close the KCl container.
19. Use the vortex mixer to vortex the conical tube for 15 seconds.
    - This thoroughly mixes the water and KCl inside the conical tube.
20. Label the lid of the conical tube with the volume and concentration “50 mL 600 mM KCl,” your initials (ex: “AB”), and the date (ex: “01/10/2021”), then return the conical tube to the rack.
21. In a lab notebook, record the number of prepared conical tubes and the solution information listed in Step 20.
22. Return the water, KCl, motorized pipette filler, and vortex mixer to their storage locations. Throw the used serological pipettes into the waste container.
23. Use paper towels to wipe down the lab bench with 70% ethanol solution.

3. The 600 mM KCl solution is being used as part of the production process. You are in charge of making batches of this solution for other team members to use. One day after making one batch of the 600 mM KCl solution, you realized you added NaCl instead of KCl for that batch.

    1. Outline your next steps to deal with this mistake.

- Remove the erroneous batch from the conical tube rack and any other communal storage locations.
- Reference my lab notebook for the number of conical tubes made in the erroneous batch (as described in Step 21 above) and count the number of conical tubes I have collected so far. This will allow me to determine the number of conical tubes my team members have and that I need to collect before the erroneous batch is completely recovered.
- Send the email (written below) to inform my team members of the batch error.
- Make a new batch as soon as possible for my team's use.
    - If this batch is being prepared the next morning, it will be labeled "50 mL 600 mM KCl , AB , 01/11/2021"
    - If this batch is being prepared the same day, it will be labeled "#2, 50 mL 600 mM KCl , AB , 01/10/2021"
- Once a new batch is complete, send a follow-up email to inform team members that a replacement batch is available. Provide the appropriate batch label and apologize again for any inconvenience this error may have caused.
- Dispose of the erroneous batch.
    - If the 600 mM NaCl can be utilized in any other experiments, relabel and reallocate the erroneous batch for those experiments. This can involve further diluting the 600 mM NaCl to another desired concentration.
    - If the 600 mM NaCl cannot be used in other, current experiments and it cannot be stored in the lab space for potential future use, dispose of the solution by either placing it in the appropriate waste container or diluting the solution with water and draining the liquid in the sink.

    2. Part of the response includes sending out an email to the team members. Please draft a one-paragraph email.

Hi all,

I just wanted to inform you that there was an error in the preparation of today's batch of 600 mM KCl solution, where NaCl was added instead of KCl. If you used 50 mL conical tubes labeled "50 mL 600 mM KCl , AB , 01/10/2021" in your experiments today, this mistake impacted your work and I sincerely apologize for any disruption this mistake had in your research. If you still have any of the conical tubes from this erroneous batch, please return them to me by either placing them in my conical tube rack or handing the tubes back to me directly. I will be preparing a new batch as soon as possible to replace today's solution, and will send out a follow-up email once the batch is complete.

Best,

Anya Bekhtel
MI | Manufacturing Associate