**Name**_____ **Period** _____ **Date** _____

# Lui Freeze-Thaw Elution

STEM Sims

**Lesson 1: Lui Freeze Thaw Elution**

Elution refers to the removal of antibodies from the surfaces of cells. The Lui freeze/thaw elution is a procedure designed to recover the antibodies bound to red blood cells when the suspected antibody is anti-A or anti-B. In this investigation, you will complete a Lui freeze/thaw elution and identify the associated antibodies collected.

**Doing the Science**

1. Open the Lui Freeze/Thaw Elution simulation.

*Part I: Preparing the Sample*

2. Select a test tube from the shelf and move it to the two-holed rack on the table.
3. Select the marker and move the marker to the test tube in the rack on the table to label the tube with the patient's ID number.
4. Move the patient's pink-capped tube on the shelf to the two-holed rack on the table.
5. Select a pipette from the shelf and move the pipette to the patient's pink-capped tube in the rack.
6. Move the pipette to the empty test tube in the two-holed rack on the table.
7. Move the pipette to the waste area.
8. Select the saline bottle from the shelf and move the bottle to the test tube on the table.
9. Move the test tube you just filled to the hopper next to the centrifuge.
10. Select an open slot in the centrifuge to load the test tube from the hopper to the centrifuge. Select a second open slot in the centrifuge to load the balance tube in the hopper to properly balance the two tubes in the centrifuge.
11. Close the centrifuge lid. Set the time for 1:00 minute, the speed on "High," and select the "Start" button.

*Part II: Washing the Sample*

12. Select a pipette from the shelf and move the pipette to the test tube in the tube holder on the table.
13. Select and move the pipette to the sink to dispense the liquid.
14. Repeat the process of adding saline, centrifuging, using the pipette to collect liquid from the test tube, and dispensing the liquid in the sink until you have completed a total of six washes (steps 8–13). **Important:** Save the liquid from the last wash. Do *not* throw away the liquid from the last wash into the sink.

*Part III: More Sample Preparation*

15. Select a new test tube from the shelf and move the tube to the two-holed rack on the table.
16. Select the marker from the shelf to label this new test tube as "Last Wash" and with the patient's ID number.

17. Select the pipette on the table and move the pipette to the test tube on the left in the tube holder containing the liquid from the six washes.
18. Move the pipette to the newly labeled "Last Wash" test tube.
19. Select the saline solution bottle on the shelf and move the bottle to the test tube in the tube holder labeled with the patient's ID number, not the "Last Wash" test tube.
20. Select the marker and move the marker to the test tube with saline on the left to add the label "Elution" to this test tube.
21. Select a piece of parafilm from the table and move the parafilm to the elution test tube in the tube holder.
22. Select and move the elution test tube to the tube roller to coat the inside of the tube with the liquid containing the cells.

*Part IV: The Freeze/Thaw Process*

23. Select and move the elution test tube to the freezer, set the timer for 10:00 minutes, and select the "Start" button.
24. Select and move the elution test tube to the sink.
25. Select and move the elution test tube to the hopper next to the centrifuge.
26. Select and move the elution test tube from the hopper to the centrifuge. Use the balance tube in the hopper to properly balance the two tubes in the centrifuge.
27. Close the centrifuge lid. Set the time for 2:00 minutes, the speed on high, and select the "Start" button.

*Part V: Preparing the Last Wash and Elution Sets*

28. Select a new pipette from the shelf and move the pipette to the "Last Wash" tube in the two-holed tube holder.
29. Select and move this pipette to each of the test tubes in the "Last Wash" tube set to dispense two drops of last wash to each test tube.
30. Select a new pipette from the shelf and move the pipette to the elution test tube in the two-holed rack.
31. Select and move the pipette to each of the test tubes in the "Elute" set to dispense 2 drops of liquid in each tube.

*Part VI: Adding Cells*

32. Select the "A cells" bottle on the shelf.
33. Move the dropper containing the A cells to the A tubes in both "Elute" and "Last Wash" tube sets to add one drop of liquid.
34. Select the "B cells" bottle on the shelf.
35. Move the dropper containing the B cells to the B tubes in both "Elute" and "Last Wash" tube sets to add one drop of liquid.
36. Select the "Screening cells I" bottle on the shelf.
37. Move the dropper containing the Screening cells I to the I tubes in both "Elute" and "Last Wash" tube sets to add one drop of liquid.
38. Select the "Screening cells II" bottle on the shelf.
39. Move the dropper containing the Screening cells II to the II tubes in both "Elute" and "Last Wash" tube sets to add one drop of liquid.
40. Select the "Screening cells III" bottle on the shelf.

41. Move the dropper containing the Screening cells III to the III tubes in both “Elute” and “Last Wash” tube sets to add one drop of liquid.

*Part VII: Incubation and Centrifuging*

42. Select the “Last Wash” set of test tubes and move the set to the dribath incubator.
43. Select the “Elute” set of test tubes and move the set to the dribath incubator.
44. Use the arrows to set the timer for 20 minutes and select the “Start” button.
45. Select the saline container from the shelf and move the saline to the “Last Wash” test tubes on the table. Please note: all the “Last Wash” test tubes in the rack will be washed simultaneously.
46. Select the saline container from the shelf and move the saline to the “Elution” test tubes on the table. Please note: all the “Elution” test tubes in the rack will be washed simultaneously.
47. Select the test tubes on the table and move the test tubes to the centrifuge hopper.
48. Close the centrifuge lid. Set the timer for 1:00 minute, the speed on high, and select the “Start” button.
49. Select a new pipette from the shelf and move the pipette to one of the “Last Wash” test tubes in the rack.
50. Select a new pipette from the shelf and move the pipette to one of the “Elution” test tubes in the rack.
51. Select the "Last Wash: and "Elution" test tubes and move them to the centrifuge hopper. The process of washing the sample with saline solution and centrifuging two more times is done automatically. Please wait until this process is completed before moving onto the next step in the procedure.

*Part VIII: Preparing the Sample for Observations*

52. Select the AHG bottle from the shelf and move the bottle to one of the test tubes on the table.
53. Select and move a test tube on the table to the Tube Roller to hand agitate the tubes.
54. Select the Last Wash and Elution test tubes on the table and move the test tubes to the centrifuge hopper.
55. Close the centrifuge lid. Set the time for 0:15 seconds, the speed on “High”, and select the “Start” button.
56. Select and move the test tubes from the hopper to the rack on the table.
57. Select one test tube from the rack and move the test tube to the agglutination viewer to analyze the sample. Record in Table 1 your observations and inferences.
58. Repeat this viewing process until all ten test tubes have been analyzed and recorded in Table 1.
59. When your observations are completed, select the “Evaluation” button at the bottom of the screen.
60. Select and record in Table 1 the reaction type for each test tube (0, 1+, 2+, 3+, or 4+). You may select an image to view the reaction tube in a larger size. Select whether the additional “Check Cells” procedure is required or not. After classifying all ten of the reaction types, select the “Check Answer” button (or “Submit Answer” button if in the test mode) to evaluate your answers.

Table 1. Sample Evaluation

| Last Wash | | | | |
|---|---|---|---|---|
| A Cells | B Cells | I Cells | II Cells | III Cells |
| | | | | |
| | | | | |
| Check Cells Procedure Required: | | Yes ☐ No ☐ | | |
| | | | | |
| Eluate | | | | |
| A Cells | B Cells | I Cells | II Cells | III Cells |
| | | | | |

**Do You Understand?**

1. If A cells have a value of 1+ and B cells have a value of 3+, what are the expected blood types of the baby and mother?

2. If A cells have a value of 2+ and B cells have a value of 1+, what are the expected blood types of the baby and mother?

3. If A cells have a value of 0 and B cells have a value of 3+, what are the expected blood types of the baby and mother?