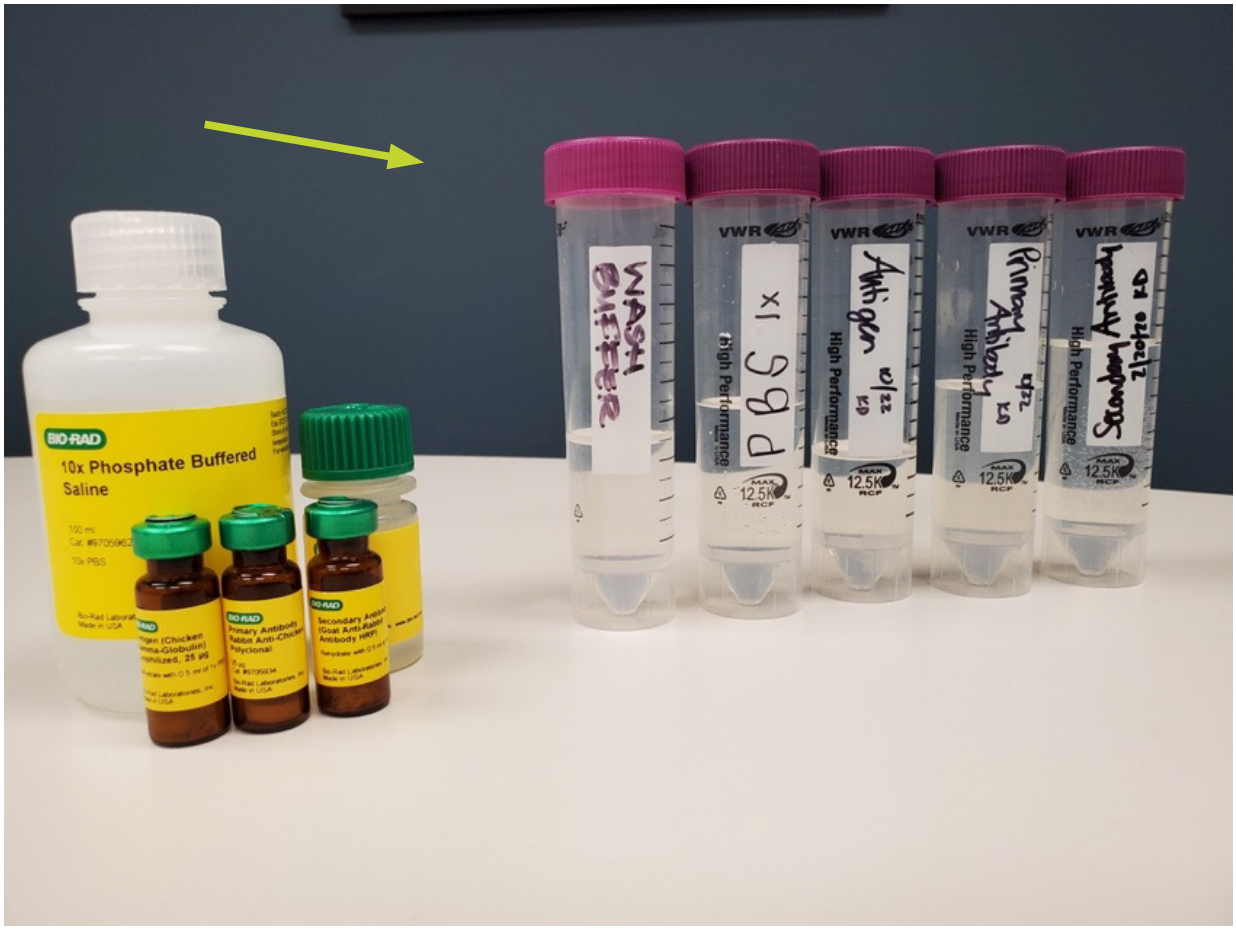
GENERATION HEALTH
How Science Powers Us

Teacher's Guide to Lab Activity

(Prepare in advance of class)

REAGENT PREP

Follow the preparation for the reagents found in the Instruction Manual provided by Bio-Rad. Upon completion of the preparation, you should have a vial/tube of 1x PBS, wash buffer, antigen, primary antibody, and secondary antibody.



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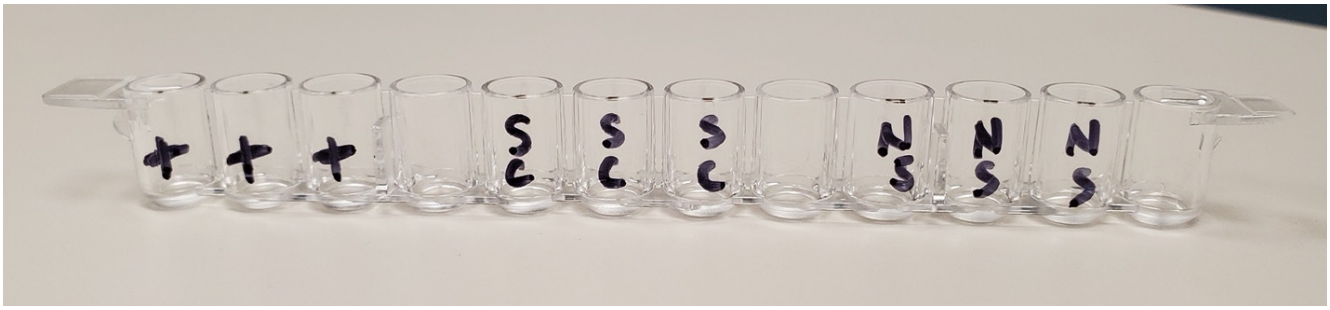
PREPARING THE WELLS

We will prepare the wells to create various outcomes, due to the multiple positive test case scenarios using only one patient sample.

Label the wells as the following:
The first three wells marked "+"
Spacer
Next three as "SC"
Spacer
Last three as "NS"



Depending on the class size, you may decide how many cases of high expressions and false positive instances you wish for students to observe in the lab activity. There should be at least one instance of a false positive (no expression of lung cancer biomarkers), one high expression of small cell lung cancer biomarkers, and one high expression of non-small cell cancer biomarkers.

False Positive (No expression of lung cancer biomarkers)
Leave all wells empty.

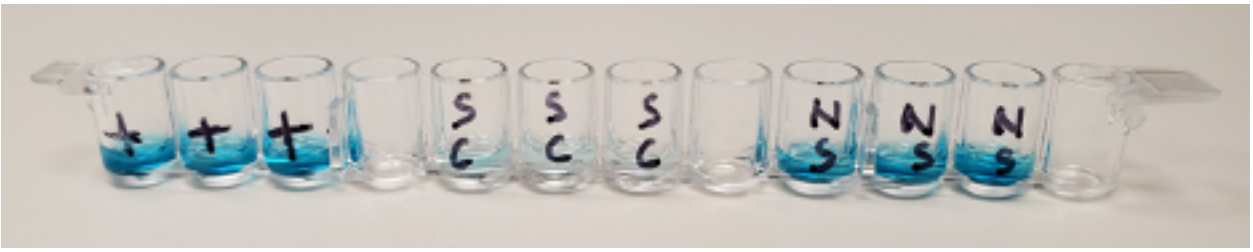
Positive Small Cell Cancer (High expression of small cell lung cancer biomarkers)
Take a fresh pipet and transfer 50 μL of the antigen to the positive well.
Transfer 50 μL of the antigen to the wells labeled "SC".
Leave the remaining wells empty.

Positive Non-Small Cell Cancer (High expression of non-small cell lung cancer biomarkers)
Take a fresh pipet and transfer 50 μL of the antigen to the positive well.
Transfer 50 μL of the antigen to the wells labeled "NS".
Leave the remaining wells empty.

Wait 5 minutes for the antigen to stick to the walls of the well.

Wash out the wells by first emptying the wells onto a paper towel. Tip over the wells and gently tap the top of them to remove remaining liquid inside the wells. Once the wells are empty, Transfer 200 µL of the washing buffer to each well. Be sure to use a fresh pipet for the washing buffer. Once all wells have been filled with the washing buffer, tip the wells over and gently tap the back of the wells to empty them. Repeat washing process one more time.

The wells are now ready to go for the student activity.



This is the outcome at the end of the investigation for the setup of high expression of non-small cell lung cancer.

WORKSTATION SETUP

Fill your colored tubes (1.5 mL) with the appropriate content. In the event you do not have colored tubes, just make sure the tubes are clearly labeled. The exception is the enzyme substrate. The enzyme substrate is LIGHT SENSITIVE and REQUIRES a dark colored tube. The content listed is enough for two runs. Multiply as needed. Wash Buffer can be put into larger vials or beakers, the listed amount is just the minimum required for two sets of wells or runs.

Item (Label) <Biomarker>	Content	Check
Yellow Tube (PS)	1x PBS (1.5 mL)	☐
Violet Tube (+) <CEA>	Primary Antibody (0.5 mL)	☐
Blue Tube (SC) <ProGRP>	Primary Antibody (0.5 mL)	☐
Green Tube (NS) <NSE>	Primary Antibody (0.5 mL)	☐
Orange Tube (SA)	Secondary Antibody (1.5 mL)	☐
Brown Tube (SUB)	Enzyme Substrate (1.5 mL)	☐
Vial or Beaker	Wash Buffer (10mL)	☐