

# Breast Cancer Biology Kit Reagent Sourcing, Storage and Preparation

## Purpose of this Guide

This document will provide you with the following information for the Influenza Outbreak Investigation Kit:

- Where to order reagents & supplies for the Breast Cancer Biology labs
- How to store the associated reagents
- How to prepare the reagents for use in the classroom
- Which consumables and equipment are needed to run the labs in this kit

## How to Use this Guide

1. Determine how much you need of supplies and reagents for the number of students/classes. There is a kit prep packing list that details how much of each reagent/consumable/equipment is needed for a class of 8 student groups. The name of this document is: Breast Cancer Biology Kit Prep Packing List.
2. Order materials needed for your labs well ahead of when you plan to use them with students.
3. When materials arrive, make sure they are stored properly. Storage information is included in this document and should also be on the packing slip.

## Kit Scenario

The curriculum scenario provides a foundation in cancer biology using a hypothetical case study of 12 fictitious breast cancer patients. Patient samples are analyzed via ELISA, PCR, bioinformatics, and fluorescent cell staining data. The ELISA test data is used to determine the probability that a patient's cancer has metastasized. PCR, bioinformatics, and fluorescent cell staining results are used to determine potential causes of each patient's cancer. Each student group tests 3 patient samples ( $8 \times 3 = 24$ ) in each lab, so each patient gets tested twice.

# Lab 1: ELISA Lab

## Lab 1 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?*
Protein A-HRP	Invitrogen	101023	-20 °C Freezer Protected from light	Yes – Needs to be diluted into ELISA Coating Buffer
ELISA Coating Buffer 0.1M Carb/Bicarb buffer pH 9.3-9.6	Make in-house or purchase from  Tribioscience (or other sources)	Na Carb ( $\text{Na}_2\text{CO}_3$ ) + Na Bicarb ( $\text{NaHCO}_3$ ) Or TBS5058-500	Refrigerator	
10X Phosphate Buffered Saline (PBS)	Fisher Scientific	BP399500 (500mL)	Room temperature	Yes – Needs to be diluted to 1X
ELISA Wash Buffer PBS-Tween (1X PBS + 0.05% Tween 20)	1X PBS (made by diluting 10X PBS) plus 0.05% Tween 20	Tween 20 from Thomas Scientific C791P51 (500mL) ELISA Wash Buffer already made from Thermo Scientific J63596.K2	Room temperature	
ELISA Substrate – TMB	Novex by Life Technologies	002023	Refrigerator Protected from light	
ELISA plates – 96 well plates that are specifically for ELISA	Many sources Ex: BioRad	2240096EDU – 100 plates, Costar 96w flat-bottom EIA plates (\$521.55) 1662405EDU – 3 plates, flat bottom EIA (kit refill) (\$38.18)	Room temperature	
1.5mL microfuge tubes	Multiple companies: Thomas Scientific, Fisher Scientific, Carolina Biological etc		Room temperature	

## Lab 1 Preparation Directions

The following reagents need to be prepped before Lab 1:

- 1) **Protein A-HRP** - Aliquot into 5uL or 10uL aliquots and store in a box in the freezer. Dilute as needed in 1X PBS.
- 2) **1X PBS** - Dilute 1 part 10X PBS with 9 parts distilled water. (Example: For 1L – 100mL of 10X PBS plus 900 mL di water)
- 3) **ELISA Coating Buffer** - Use on-line calculator to determine how much Na Carb and Na Bicarb to use for the desired volume and pH. <https://www.aatbio.com/resources/buffer-preparations-and-recipes/carbonate-bicarbonate-buffer-ph-9-2-to-10-6>  
You can also purchase it already made.
- 4) **ELISA Wash Buffer** – See recipe in **Appendix A**

## Lab 2: PCR and Gel Electrophoresis Lab

### Lab 2 Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?*
Positive control DNA (Use at 2.5ng/uL)	Raspberry-H1-pET21a plasmid		-20 °C Freezer	Yes – Needs to be diluted to 20ng/uL and mixed with the appropriate R primer (10uM stock) before class
negative control – ie no DNA (sterile di water)	Make in-house or purchase	Sigma, Gibco etc	Room Temperature	
HER2 Forward primer (Lac-O-RBS) Use at 10uM	Integrated DNA Technologies (IDT)		Lyophilized primers – Room Temperature  100uM stock solutions – -20 °C Freezer	Yes – Needs to be reconstituted with diH2O to 100uM and stored. Needs to be diluted to 10uM before class
HER2 Reverse primer RH1-R1 For generation of 1,000 bp (1 copy) band Use at 10uM	IDT		Lyophilized primers – Room Temperature	Yes – Needs to be reconstituted with diH2O to 100uM and stored. Needs to be diluted to 10uM before class and

			100uM stock solutions – -20 °C Freezer	mixed with the template DNA
HER2 Reverse primer RH1-R4 For generation of 2,000 bp (2 copy) band Use at 10uM	IDT		Lyophilized primers – Room Temperature  100uM stock solutions – -20 °C Freezer	Yes – Needs to be reconstituted with diH2O to 100uM and stored. Needs to be diluted to 10uM before class and mixed with the template DNA
HER2 Reverse primer RH1-R3 For generation of 3,000 bp (3 copy) band Use at 10uM	IDT		Lyophilized primers – Room Temperature  100uM stock solutions – -20 °C Freezer	Yes – Needs to be reconstituted with diH2O to 100uM and stored. Needs to be diluted to 10uM before class and mixed with the template DNA
One Taq Quick Load 2X Master Mix with Buffer	NEB	m0271S	-20 °C Freezer	
Agarose	Thomas Scientific	C748D75	Room Temperature	Yes – needs to be melted in 1X TAE and gelgreen added to pour gels before class or during class
50X TAE	Thomas Scientific	B49	Room Temperature	Yes - Can buy or make 50X TAE, needs to be diluted to 1x before class
1 kb DNA Ladder	NEB	N3232S	-20 °C Freezer	
GelGreen	Biotium	41005	Room Temperature Protected from light	
6X Loading Dye	NEB	B7025S	-20 °C Freezer	
1.5mL microfuge tubes	Multiple companies: Thomas Scientific, Fisher Scientific, Carolina Biological etc		Room temperature	

PCR tubes	Any scientific supply company – Thomas Scientific, Fisher Scientific, Carolina Biological etc		Room temperature	
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\* See prep directions in following section

## Lab 2 Preparation Directions

The following reagents need to be prepped before Lab 2:

- 1) HER2 positive DNA** - Raspberry-H1-pET21a plasmid – Dilute to 20 ng/uL with sterile di water.
- 2) Forward and Reverse Primer 100uM stocks** - – Reconstitute the lyophilized primers to 100uM with sterile di water. The IDT information sheet that comes with each primer will tell you how much water to add to the tube of lyophilized primer to make a 100uM solution. Keep this 100uM primer solution as a 10x stock solution. Store frozen.
- 3) DNA Template + R Primer Solutions**  
To control the size of the DNA band amplified and not give away which patients will have which results, the DNA template is mixed with the appropriate reverse primer and then aliquoted and labeled by patient or + control (we use the HER2 2 copy DNA/R4 primer mix as the positive control). The negative control contains water instead of DNA/R primer. The following mixtures make enough sample for a kit for **one class of 8 student groups** plus enough for a couple of extra reactions.

### DNA Template and R primer solutions

(+1) One copy of HER2 – (20 reactions)	
Reagent	Volume
DNA template (20ng/uL)	22 uL (1uL/rxn)
R1 primer (10uM)	55 uL (2.5uL/rxn)
Sterile distilled water	143 uL (6.5uL/rxn)
Total volume	220 uL (10uL/rxn)

(+2) Two copies of HER2 – (12 reactions)	
Reagent	Volume
DNA template (20ng/uL)	12 uL (1uL/rxn)
R4 primer (10uM)	30 uL (2.5 uL/rxn)
Sterile distilled water	78 uL (6.5uL/rxn)
Total volume	120 uL (10uL/rxn)

(+3) Three copies of HER2 – (6 reactions)	
Reagent	Volume
DNA template (20ng/uL)	6 uL (1uL/rxn)
R3 primer (10uM)	15 uL (2.5uL/rxn)
Sterile distilled water	39 uL (6.5uL/rxn)

Total volume	60 uL (10uL/rxn)
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Negative control = 100uL sterile distilled water

This is enough for 10 negative control reaction (10uL per reaction)

Primer 'cocktail' = 130uL of LacO-to-RBS-12 primer at 10uM. This 'primer cocktail' contains only the forward primer. This way all students receive the same primer cocktail. We do not explain to the kids the fact that the R primer is mixed in with the DNA template.

This is enough for all 40 reactions (plus a little extra) at 2.5uL per reaction. The reactions include:

- 24 patient reactions (A-L x 2 – ie A.1 and A.2, B.1 and B.2 etc)
- 8 positive control reactions
- 8 negative control reactions

- 4) Forward Primer 10uM Solution** – Create a solution in which the forward primer is at a concentration of 10uM. 130uL of LacO-to-RBS-12 primer at 10uM. This 'primer cocktail' contains only the forward primer. This way all students receive the same primer cocktail. We do not explain to the kids the fact that the R primer is mixed in with the DNA template.
- a. For 130uL of a 10uM solution, put 117uL of sterile di water into a microfuge tube.
  - b. Add 13uL of the 100uM solution of the reverse primer and mix well.
  - c. Store frozen.

**NOTE:** The recipes in #3 and #4 make enough for all 40 reactions needed by a class with 8 student groups (plus a little extra) at 2.5uL per reaction. The reactions include:

- 24 patient reactions (A-L x 2 – ie A.1 and A.2, B.1 and B.2 etc)
- 8 positive control reactions
- 8 negative control reactions

#### Primer Sequence Information

Primer Name	Type – Forward or Reverse	Sequence 5' to 3'
LacO-to-RBS-12	Forward	CTC TAG AAA TAA TTT TGT TTA ACT TTA AGA AGG AG
RH1-R1	Reverse	GAG TTT TCC ATT ATG GTT GTC TTC G
RH1-R4	Reverse	ACT GAG TTC ACC TTG TTG GTT ATC C
RH1-R3	Reverse	GGA ACA AGA GTC CAC TAT TAA AGA ACG

- 5) 1X TAE** – This solution is used to make agarose gel and as running buffer. It is made by diluting 50X TAE to 1X with distilled (di) water. 50X TAE can be made in house or purchased. To make 50X TAE in house, see the following protocol at: <https://www.protocols.io/view/recipe-for-50x-tae-buffer-ewov1d47vr24/v1>

- a. For 1 Liter of 1X TAE, mix 20mL of 50X TAE with 980mL of di water.
  - b. Mix well.
  - c. Store at room temperature.
- 6) **GelGreen** - GelGreen is diluted into the melted agarose gel mix at 1:10,000 (1uL of GelGreen stock into 10mL of liquid gel). Mix well by swirling before pouring into gel casting stand.
- 7) **Gels** – Pour as many gels as needed in the 0.75% - 1% range.
  - a. Put the desired amount of 1X gel running buffer in an Erlenmeyer flask. Make sure the solution does not fill the flask. Ex: for 50mL of gel, use a 250mL flask
  - b. Weigh out the appropriate amount of agarose.
  - c. Add the agarose to the running buffer and swirl to mix.
  - d. Microwave on high until all agarose crystals are melted. Stop and swirl and check every 30 sec or so. Don't let it boil over. Use a hot pad when handling the hot flask.
  - e. Add GelGreen at 1uL/10mL of liquid gel.
  - f. Allow the solution to cool a little, so it's no longer boiling hot.
  - g. Pour into casting stands that have combs in them.
  - h. The gels can be stored up to a week if kept moist (in ziplock bag) and in the dark and cold (refrigerator).

**NOTE:** You do not need to use the Raspberry-H1-pET21a plasmid for this lab. You can use any plasmid you have available. If you are using a different plasmid, you need to design a forward primer and three reverse primers that will amplify fragments of nested sizes. If the size of the amplicons is different than what is described in the protocol for this kit, you will need to change some of the text etc in the protocol document. The curriculum for this kit describes fragments of 1kb, 2kb, and 3kb. You could instead create fragments of 0.5kb, 1.0kb, and 1.5kb for instance.

## Lab 3: BRCA1 Bioinformatics Lab

### Lab 3 Ordering Information

Nothing to order. All Links to data are available on the Canvas page.

## Lab 4: Estrogen Receptor Fluorescent Staining Lab

### Lab 4 Ordering Information

Nothing to order. Fluorescent staining images are available on the Canvas page.

# Antibody Modeling Activity

## Antibody Modeling Ordering Information

Name	Source	Cat or Model #	Storage	Requires prep before class?*
Colored chenille stems (pipe cleaners)	Any craft store or online		Room temperature	
Black chenille stems (pipe cleaners)	Any craft store or online		Room Temperature	It saves time if you cut black stems into 5-6 pieces before class
Perler beads or any other type of bead that a chenille stem can fit through	Any craft store or online		Room Temperature	

## Equipment Required for Breast Cancer Kit

Name	Source	Example Source Cat or Model #
Micropipette tips P20 P200 P1000	Any scientific supply company	Thomas Scientific, Fisher Scientific, Carolina Biological etc
Gel Boxes	Multiple sources	EmbiTec – MiniOne (has LED) MiniPCR – (has LED) BioRad etc
Blue LED light boxes – if LED light is not built into your gel boxes	Multiple sources	
Micropipettes P20 P200 P1000	Multiple sources	Rainin, Gilson etc
Mini Microfuges	Multiple sources	
Tube racks	Multiple sources	
Thermocycler (PCR Machine)	Multiple sources	BioRad T100, EmbiTec-MiniOne, MiniPCR
Ice buckets or styrofoam containers	Any scientific supply company	
Microwave oven		



