# Influenza Outbreak Investigation 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 Influenza Outbreak Investigation 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 prep key that details how much of each reagent/consumable/equipment is needed for a class of 8 student groups. The name of this document is: Influenza Outbreak 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 is an outbreak of influenza among a fictitious group of people who have all been on the same airline flight. The curriculum includes 32 Patient Cards that contain information regarding: age, date of last influenza vaccine, height, weight, blood pressure, symptoms, etc. These cards have a numbered patient ID on them to protect the privacy of each individual. Patient samples are analyzed via ELISA, PCR, and Bioinformatics.

# 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<br><br>Tribioscience | Na Carb ($Na_2CO_3$) + Na Bicarb ($NaHCO_3$) Or<br>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)<br>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)<br>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 | |

** See prep directions in following section*

## 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?*** |
|---|---|---|---|---|
| Flu positive DNA (Use at 2.5ng/uL) | N1-Sweden-pET21a plasmid | | -20 °C Freezer | Yes – Needs to be diluted to 2.5ng/uL before class |
| Flu negative DNA (sterile di water) | Sterile di water | | Room Temperature | |
| Influenza Neuraminidase Forward primer (Lac-O-RBS) Use at 10uM | Integrated DNA Technologies (IDT) | | Lyophilized primers – Room Temperature<br><br>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 |
| Influenza Neuraminidase Reverse primer (T7 Terminator) Use at 10uM | IDT | | Lyophilized primers – Room Temperature<br><br>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 |

| | | | | |
|---|---|---|---|---|
| One Taq Quick Load 2X Master Mix with Buffer | NEB | m0271S | -20 °C Freezer | |
| Sterile distilled water | Make in-house or purchase | Sigma Gibco etc | Room temperature | |
| 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 | |

** See prep directions in following section*

## Lab 2 Preparation Directions

The following reagents need to be prepped before Lab 2:

1) **Flu Positive DNA -** N1-Sweden-pET21a plasmid – Dilute to 2.5 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) **Forward and Reverse Primer 10uM PCR Mixture –** Create a solution in which each primer is at a concentration of 10uM.
    a. For 100uL of a 10uM solution, put 80uL of sterile di water into a microfuge tube.
    b. Add 10uL of the 100uM solution of the reverse primer and mix well.

c. Add 10uL of the 100mM solution of the forward primer and mix well.
d. Store frozen.

**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 |
| T7-Term-15 | Reverse | CCC CAA GGG GTT ATG CTA G |

4) **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.
5) **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.
6) **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 N1-Sweden-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 and a reverse primer that will amplify a fragment of a desired size. If the size of the amplicon 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.

# Lab 3: Influenza Bioinformatics Lab

## Lab 3 Ordering Information

Nothing to order. All Links to data 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 the Influenza Outbreak Kit

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