**BIOTECH Project Resource Center Loan Checklist**

Teacher: Date loaned:

School: # groups:

students/classes: Independent:

**Materials for ELISA (students work in groups of 3)**:

|  |  |  |
| --- | --- | --- |
| Number | Item | Returned? |
| 1 per student | body fluid tubes (labeled BF) |  |
| 1 per class | disease body fluid tubes- (labeled 'BF' with piece of yellow tape)  this is the one person in the class that has the 'disease' |  |
| 1 dropper  bottle per  class | positive control (+) (10 ml per bottle)  If only 1 class is doing the lab, divide the contents of the dropper bottle  between two bottles |  |
| 1 dropper  bottle per  class | negative control tubes (-) (10 ml per bottle)  If only 1 class is doing the lab, divide the contents of the dropper bottle  between two bottles |  |
| 1 dropper  bottle per  class | Ab tubes (15 ml per bottle)  If only 1 class is doing the lab, divide the contents of the dropper bottle  between two bottles |  |
| 1 dropper  bottle per  class | TMB tubes (15 ml per bottle)  If only 1 class is doing the lab, divide the contents of the dropper bottle  between two bottles |  |
| 1 per student | large non sterile transfer pipets |  |
| 1 per group | 24 well plates |  |
| 3-4 | wash bottles half-full with wash solution |  |

When you are reloading the bins, please check off each item in the 'Returned?' column as a double-check that all those little pieces of equipment get packed. Thanks!

If you have questions about experiments or materials, please feel free to contact the BIOTECH Project at:

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**Have fun!**

**• Body fluid and negative control solution = 1X Na2CO3 buffer ( you will need approximately 5 mls per class, so make 10 ml for 1-2 classes, 20 mls 3-4 classes and 30 ml for 5-6 classes.**

10X Stock solution of Na2CO3

3.2 g Na2CO3

5.86 g NaHCO3

Dissolve in enough water to make a total volume of 200 ml. This can be stored indefinitely in the refrigerator.

1X Na2CO3 = body fluid or negative control

Combine 20 ml 10X stock and 180 ml distilled water. Pipet into 1 ml aliquots and store in the refrigerator. Label 'BF'' for body fluid. Add the rest to Negative Control dropper bottle.

**• Positive body fluid and positive control solution = 1X Na2CO3 buffer + BSA ( you will need approximately 5 mls per class, so make 10 ml for 1-2 classes, 20 mls 3-4 classes and 30 ml for 5-6 classes.**

Biotinylated BSA solution

Dissolve 10 mg biotinylated BSA (bovine serum albumin) in 20 ml Na2CO3 buffer. Store at -20°C in 1 ml aliquots.

BSA solution

Dissolve 10 mg BSA in 10 ml Na2CO3 buffer. Pipet into 1 ml aliquots and store at -20°C.

Positive BF and positive control solution

0.1 ml (100 l) biotinylated BSA solution

9.85 ml 1X Na2CO3

50 µl BSA solution

Mix, and pipet into 1 ml aliquots (label with 'BF' with a piece of yellow tape,) store in refrigerator for up to one week. The rest will be added to Positive Control Dropper bottle.

**• Antibody solution**

Streptavidin peroxidase stock solution

1 ml 50% glycerol

0.5 mg streptavidin peroxidase (this amount comes in container)

This solution is stable in refrigerator for several years.

20X PBS (for 1 liter)

160 g NaCl

4 g KCl

22.4 g Na2HPO4

4 g KH2PO4

Dissolve in enough water to make a final volume of one liter. Store at 4°C

Antibody solution—make enough for 15 ml per class

0.75 ml (750 l) 20X PBS \*\*\*always check for precipiate and allow crystals to redissovle before using

75 µl BSA solution

14.175 ml distilled water

Mix, then add 1.5 µl streptavidin peroxidase solution. Add to Antibody labeled dropper bottles, and store in refrigerator. Use within one week.

**• Color reagent solution (TMB)**

TMB solution

Use ready to use TMB component substrate Product no. TMBW-0100-01 or TMBC-0100-01 (vendor BioFX laboratories purchased from VWR)—long shelf life store at 4°C.

**• Wash solution (1X PBS with 0.1% Tween-20)**

50 ml 20X PBS \*\*\*always check for precipiate and allow crystals to redissovle before using

950 ml distilled water

10 ml 10% Tween-20

Store at room temperature.