**Vero Cell Cytotoxicity Calculation Worksheet**

**Counting the Vero Cells**

5.2.4) Calculate the average number of viable Vero Cells.

Quadrant 1: \_\_\_\_\_\_\_\_ cells Sum of Quadrants: \_\_\_\_\_\_\_\_\_ cells

Quadrant 2: \_\_\_\_\_\_\_\_ cells

Quadrant 3: \_\_\_\_\_\_\_\_ cells Average: Sum/4 = **\_\_\_\_\_\_\_\_\_\_ cells**

Quadrant 4: \_\_\_\_\_\_\_\_ cells

5.2.5 and 5.2.6) Multiply by dilution factor (2) and correction factor (104 for hemocyometers).

Density = # cells/mL = Average cells x 2 x 104 = \_\_\_\_\_\_\_\_ x 2 x 104 = **\_\_\_\_\_\_\_\_ cells/mL**

5.2.7) Multiply by total volume to give total number of cells.

**Total # cells** = (# cells/mL) (total volume of cell suspension)

­­­ = \_\_\_\_\_\_\_\_\_\_\_ x \_\_\_\_\_\_\_\_\_\_\_ = \_\_\_\_\_\_\_\_\_\_ **total cells**

**Determining number of wells required for each experiment**

5.3.1) Determine how many wells that can be utilized based of amount of Vero Cells available.

**Total # cells**  = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **mL**

(105 cells/0.090 mL) (105 cells/0.090 mL)

# wells = ­­\_\_\_\_\_\_\_\_\_ mL = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **wells**

0.090 mL

5.3.2) Determine how much volume of Vero cell suspension needed to fill the number of desired wells.

\_\_\_\_\_\_\_\_\_\_\_\_ wells x 0.090 mL/well = \_\_\_\_\_\_\_\_\_\_\_\_\_ mL required

**\*\*\*\*\*\* Add in extra to account for pipetting error \*\*\*\*\*\***

Round up at least 1 mL extra = ­­­\_\_\_\_\_\_\_\_\_\_\_\_\_ **mL required**

5.3.3) Calculate the dilution of the Vero Cell suspension required.

C1V1 = C2V2

# cells x (V1) = 105 cells x (mL required)

 mL 0.090 mL

V1 = 105 cells x (mL required) x ( 1 )

 0.090 mL (cells/mL)

V1 = 105 cells x (\_\_\_\_\_\_\_\_\_\_\_\_ mL required) x ( 1 )

 0.090 mL \_\_\_\_\_\_\_\_\_\_\_\_\_(cells/mL)

V1 = \_\_\_\_\_\_\_\_\_\_\_\_\_ **mL of stock Vero Cells into** ­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **mL DMEM media**

**Creating stock solutions from samples (feces or intestinal content)**

5.5.1) Calculate the weight of the contents.

Final Tube Weight – Tube Weight = Content Weight

­­\_\_\_\_\_\_\_\_\_\_\_\_ g – \_\_\_\_\_\_\_\_\_\_\_ g = **\_\_\_\_\_\_\_\_\_\_\_\_\_ g**

5.5.2) Convert grams to mg to μL.

(g content) (103 mg/g) (μl/mg) = \_\_\_\_\_\_\_\_\_\_\_\_ g x 103 = \_\_\_\_\_\_\_\_\_\_\_\_\_ **μl content**

5.5.3) Calculate the final total number of μl solution needed to make a 1:10 dilution in 1X PBS.

(μl content x 10) = \_\_\_\_\_\_\_\_\_\_\_\_ μl x 10 = \_\_\_\_\_\_\_\_\_\_\_\_\_ **μl solution**

5.5.4) Calculate the total amount of 1X PBS to add to the sample.

(μl solution) – (μl content) = \_\_\_\_\_\_\_\_ μl – \_\_\_\_\_\_\_\_\_ μl content = \_\_\_\_\_\_\_\_\_ **μl PBS**

**Creating Dilution Plate #2**

 5.7.2) Calculate how much antitoxin is required for this assay.

20 μl x (6 wells) x (# rows) = 20 x 6 x \_\_\_\_\_\_\_\_\_ = \_\_\_\_\_\_\_\_\_\_ μl of 1X antitoxin

\*\* Round up to the nearest hundred to account for pipetting error\*\*\*

 = \_\_\_\_\_\_\_ **μl antitoxin needed**

Antitoxin is 25X stock, thus needs to be diluted 1:25 in 1X PBS.

(Amount of antitoxin needed/25)

\_\_\_\_\_\_\_\_μl/25 = \_\_\_\_\_\_\_\_\_\_\_\_ μl 25X stock antitoxin

\_\_\_\_\_\_\_\_ μl antitoxin needed – \_\_\_\_\_\_\_\_\_\_ μl 25X stock = \_\_\_\_\_\_\_\_**μl PBS to add**

**Plate Maps**

**Dilution Plate #1 (DP#1)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dilution | 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 | 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F |  |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

**Dilution Plate #2 (DP#2)**

 **20 μL sample + 20 μL PBS 20 μL sample + 20 μL Antitoxin**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dilution | 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 | 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F |  |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

**Vero Cell Plate**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Dilution | 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 | 10-1 | 10-2 | 10-3 | 10-4 | 10-5 | 10-6 |  |  |
| ID |  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |  | ID |
|  | Sample | A |  |  |  |  |  |  |  |  |  |  |  |  | Sample |  |
|  | AT | B |  |  |  |  |  |  |  |  |  |  |  |  | AT |  |
|  | Sample | C |  |  |  |  |  |  |  |  |  |  |  |  | Sample |  |
|  | AT | D |  |  |  |  |  |  |  |  |  |  |  |  | AT |  |
|  | Sample | E |  |  |  |  |  |  |  |  |  |  |  |  | Sample |  |
|  | AT | F |  |  |  |  |  |  |  |  |  |  |  |  | AT |  |
|  | Sample | G |  |  |  |  |  |  |  |  |  |  |  |  | Sample |  |
|  | AT | H |  |  |  |  |  |  |  |  |  |  |  |  | AT |  |

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**Dilution Plate #1 (DP#1)**

**Dilution Plate #2 (DP#2)**

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**Vero Cell Plate**