

13th February 2007

DILUTION OF MSP3 IN PBS COATING BUFFER

- The antigen (MSP3) is supplied at 0.3 mg/ml and the required (working) concentration is 0.5 µg/ml.
- Coating is done using 100 µl (of MSP3 in PBS coating buffer) per well.
- One plate has 96 wells but the first two columns i.e 16 wells are coated with serially diluted standard reference antibody (standard proteins) thus remaining 80 wells to be coated with the antigen
- Therefore total volume per plate is 80 X 100 µl =8000 µl (8 ml).
- **Mathematics:**

$C_1V_1=C_2V_2$ where, C1= Initial concentration, 0.3 mg/ml=300µg/ml
C2=Final concentration, 0.5 µg/ml
V1=Initial volume (stock), Unknown
V2=Final volume, 8,000 µl

$$V1 = \frac{C_2V_2}{C_1} = \frac{0.5 \mu\text{g/ml} \times 8,000 \mu\text{l}}{300 \mu\text{g/ml}}$$
$$=13.33 \mu\text{l}$$

Therefore, to prepare MSP3 dilution for one plate one should pipette 13.33 µl of MSP3 from the stock and dilute with 7986.67 µl of PBS Coating Buffer
In order to compensate for any spillages and losses during the process some additional volume may be needed.

We planned to coat four sets for each of the 6 antibody types (IgG, IgG1, IgG2, IgG3, IgG4 and IgM) and this makes a total of 4 X 6 = 24 plates

- **Mathematics:**

100 µl X 80 X 24 =192,000 µl i.e 192 ml

To compensate for loss the volume has been adjusted to 200ml.

$C_1V_1=C_2V_2$ where, C1= Initial concentration, 0.3 mg/ml=300µg/ml
C2=Final concentration, 0.5 µg/ml
V1=Initial volume (stock), Unknown
V2=Final volume, 200,000 µl

$$\frac{V1=C2V2}{V1} = \frac{0.5 \mu\text{g/ml} \times 200,000 \mu\text{l}}{300 \mu\text{g/ml}}$$
$$= 333.33 \mu\text{l}$$

This 333.33 μl of MSP3 stock was diluted in 199666.67 μl of PBS Coating Buffer