POPULATION ASSAY:
PROAMP

LOT#: _____ ORGANISM: _____

Fill Volume: _____ ml POPULATION LEVEL: _____

Performed By: _____ Date: _____

PROCEDURE:

1.0 Aseptically wipe down 5 ProAmps with 70% Isopropyl alcohol and place in sterile 50mL conical tube. Using a sterile pestle or other suitable device, aseptically crush the ampoules. Aseptically withdraw 1.0mL of the pooled solution and place into a sterile screw-capped 10mL test tube containing 9.0mL of sterile, processed water.

2.0 Heat shock in a water bath (10 minutes at 80º-85ºC for mesophiles and 15 minutes at 95º - 100ºC for thermophiles). Immediately cool in a water bath of 0º - 4ºC.

Start Time/Temperature:_____/_____ºC End Time:_____

3.0 Vortex the tube for 15-20 seconds.

4.0 Perform serial dilutions by pipetting out 1.0 mL of the aliquot into another sterile, screw-capped 10 mL test tube containing 9.0 mL of sterile, processed water. Repeat from step 3 until desired dilution factor is reached.

5.0 At the dilution expected to yield 10-300 CFU, pipette out 1.0mL into each of three Petri plates. Repeat for the final dilutions.

6.0 Within 20 minutes, add approximately 20 mL TSA, pre-sterilized and cooled to 47º $\pm$ 2ºC. Swirl to distribute spores evenly in agar and allow to solidify.

TSA Lot # _____ TSA Temperature:_____ºC

7.0 Invert and incubate the plates (30º-35ºC for mesophiles, 55º-60ºC for thermophiles).

Incubation Start Time:_____ Incubator #:_____

8.0 Examine all plates at 24 ($\pm$1) hours. Record on the back the number of colony forming units (CFU's) per plate. Record the average on the following page.

9.0 Calculate the average number of CFUs/ampoule from the above data using the formula on the following page.

Performed By:_____Date:_____

POPULATION ASSAY:
PROAMP

Total @ 24 hrs / number of plates counted x DF x AVF = CFU/ampoule
DF= Dilution factor (absolute value of the reciprocal of the dilution)
AV= Average number of colonies per ampoule
AFV=Ampoule fill volume

Incubation End Time: _____

CFU COUNTS AT 24 HOURS

dilutions _____

Plates 1._____ 2._____ 3._____ Total @ 24hours: _____

Total @ 24 hrs_____ / 3 x _____(DF) x _____(AFV) =_____(AV)CFU/ampoule

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CFU COUNTS AT 24 HOURS

dilutions _____

Plates 1._____ 2._____ 3._____ Total @ 24 hours: _____

Total @ 24 hrs_____ / 3 x _____(DF) x _____(AFV)=_____(AV)CFU/ampoule

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# of Dilutions = Dilution Factor
1 = 10
2 = 100
3 = 1000
4 = 10000
5 = 100000
6 = 1000000

Sum of the AV of both dilutions / 2 =CFU/ ml

_____ / 2 =

_____x10 ___ CFU/ampoule

Read By:_____Date:_____