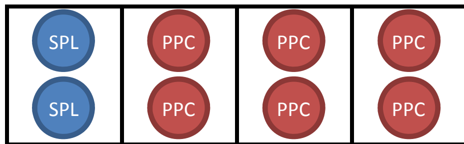
Pyrosate® CSE Quick Guide

Associates of Cape Cod, Inc.
Your Endotoxin & Glucan Experts

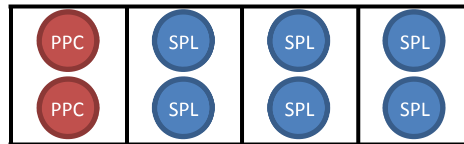
Schematic for Sample Characterization Using Pyrosate® PPC Vials

1. **Sample Characterization should be performed in duplicate together with a standard curve test in duplicate.**

2. **Set Up (Standard Curve)**
   a. Set up two racks of SPL and PPC vials in duplicate according to diagram below.



RACK 1 – Reconstitution Rack

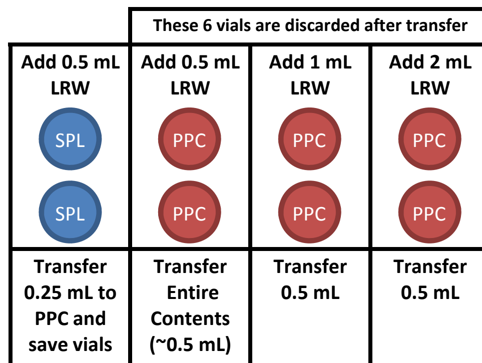


RACK 2 – Incubation Rack

3. **Preparation**
   a. Use a new pipette (or tip) for each transfer/removal combination.
   b. Remove stoppers taking care not to contaminate the vials and test per instructions below.

   c. Reconstitution

   d. Transfer



RACK 1 – Reconstitution Rack

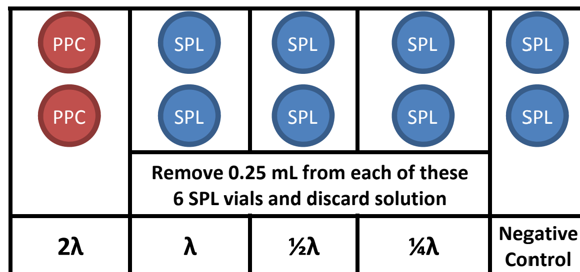
Move

Ensure contents of SPL vials are completely dissolved, then shake rack vigorously for 20-30 seconds.

After transfer of 0.25 mL to 2λ PPC, move vials to incubation rack. These are the negative controls.

Reaction starts as soon as SPL and PPC contents are combined. Perform transfers to initiation of incubation within ten minutes.

   e. Remove



RACK 2 – Incubation Rack

Ensure contents of SPL vials are completely dissolved, then shake rack vigorously for 20-30 seconds.

4. **Testing**
   a. Incubate Rack 2 in a water bath equilibrated at 37 ±1°C for the specified incubation time (± 1 minute).
   b. At the end of the incubation time, read the test by inverting each vial in one smooth motion starting with the negative control vials then ¼λ vials, etc.

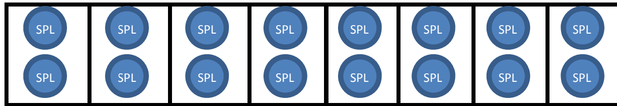
Positive test

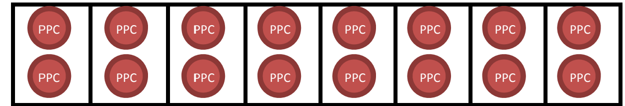
If a firm gel forms that withstands inversion, the test is scored as positive (+). All other results are negative (-), even if it is clear that a gel has formed but the clot breaks.

Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive, East Falmouth, MA 02536 USA | 888-395-2221 | www.acciusa.com

5. Set Up (Serial dilution of the sample)

a. Set up two racks of SPL and PPC vials in duplicate according to diagram below.



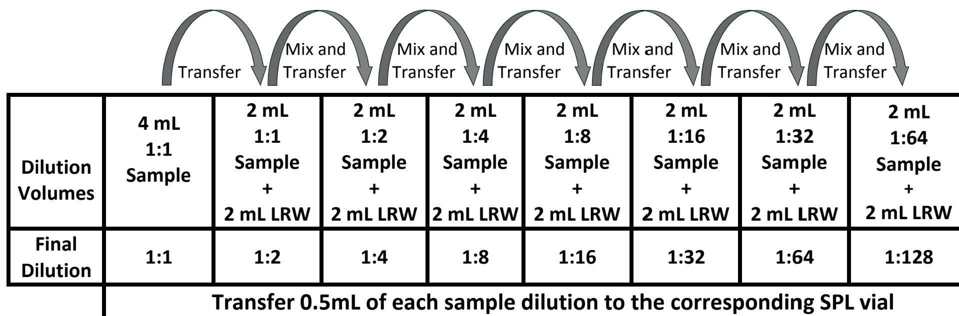


6. Preparation

a. Use a new pipette (or tip) for each transfer/removal combination.

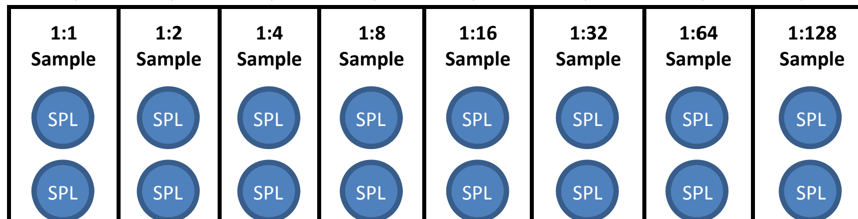
b. Remove stoppers from SPL and PPC vials just prior to reconstitution taking care not to contaminate the vials and test per instructions below.

c. Prepare seven serial twofold dilutions of test sample with LRW (1:1 = undiluted) in dilution tubes (see below).



Dilution Volumes	4 mL 1:1 Sample	2 mL 1:1 Sample + 2 mL LRW	2 mL 1:2 Sample + 2 mL LRW	2 mL 1:4 Sample + 2 mL LRW	2 mL 1:8 Sample + 2 mL LRW	2 mL 1:16 Sample + 2 mL LRW	2 mL 1:32 Sample + 2 mL LRW	2 mL 1:64 Sample + 2 mL LRW
Final Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
	Transfer 0.5mL of each sample dilution to the corresponding SPL vial							

d. Reconstitution

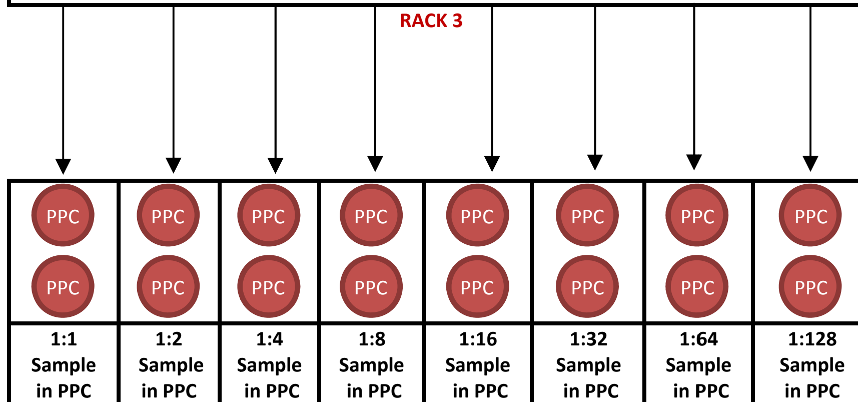


Ensure contents of SPL vials are competely dissolved, then shake rack vigorously for 20-30 seconds.

e. Transfer

RACK 3

Reaction starts as soon as SPL and PPC contents are combined. Perform transfers to initiation of incubation within ten minutes.



RACK 4

Ensure contents of PPC vials are completely dissolved, then shake rack vigorously for 20-30 seconds.

7. Testing

a. Shake Rack 3 and put in a water bath equilibrated at 37 ± 1°C. Then immediately shake Rack 4 and also put in the water bath. Incubate both racks for the specified incubation time (± 1 minute).

b. At the end of the incubation time, read the test by inverting each vial in one smooth motion starting with the highest dilution SPL and PPC vials.

If a firm gel forms that withstands inversion, the test is scored as positive (+). All other results are negative (-), even if it is clear that a gel has formed but the clot breaks.

8. Interpretation of Results of a Sample Characterization

a. Verify test validity. The negative controls should both test negative and the sensitivity of the lysate reagent (λ) should be confirmed (i.e. the geometric mean endpoint of the standards must be between ½λ and 2λ) for the standard curve. If these conditions are not met, the test is invalid.

b. If the undiluted sample (1:1) and corresponding PPC both clot, the sample contains endotoxin and the sample does not cause inhibition.

c. If the undiluted sample (1:1) does not clot, and the corresponding PPC clots, there is no endotoxin in the sample and no sample inhibition.

d. If the undiluted sample (1:1) does not clot and the PPC for the undiluted sample does not clot, the sample causes inhibition. In that case the PPC corresponding to the lowest dilution which clots represents the minimum dilution at which sample inhibition is overcome.

e. Choose a dilution of sample at least twofold higher than the minimum dilution at which sample inhibition is overcome or endotoxin is detected, which does not exceed the MVD to perform the Test for Interfering Factors.

For example: A sample has an MVD of 100-fold and the results from testing the PPC are as shown below.

PPC 1:1	PPC 1:2	PPC 1:4	PPC 1:8	PPC 1:16	PPC 1:32	PPC 1:64	PPC 1:128
-	-	-	+	+	+	+	+
-	-	-	+	+	+	+	+

In this case, the undiluted sample in PPC does not clot indicating the sample causes inhibition. Therefore the lowest concentration at which the PPC clots is the minimum dilution at which the sample inhibition is overcome. According to the results above, that dilution is 1:8. In order to perform the Test for Interfering Factors, a dilution that is at least two fold higher than the minimum dilution that overcomes inhibition should be selected. In the example above the dilution selected can be in the range 1:16 to 1:100 (the MVD). Hence, the 1:128 dilution cannot be selected.

MVD = maximum valid dilution is defined as the greatest dilution at which the endotoxin limit for the sample can be detected.

$$\text{MVD} = \frac{(\text{Endotoxin Limit})\ (\text{Concentration of Sample})}{\lambda}$$

where λ is the sensitivity of the LAL reagent in EU/mL and the endotoxin limit is expressed in EU/unit of sample (e.g. EU/mg, EU/mEq or EU/mL) and the concentration is units of sample/mL. Endotoxin limits are given in pharmacopeia monographs or may be calculated (or verified) using the information in the USP BET chapter.