

## Preparing Additional Dilutions

The purpose of a dilution is to decrease the concentration of a substance in a sample to a useful level. This is done by adding a known amount of sample to a known amount of Preparation Reagent.

Dilution calculations depend on two rules:

### RULE #1

The dilution is the amount of sample in relation to the total amount of material present.

$$D = X:(X + Y)$$

D = dilution

X = amount of sample

Y = amount of Preparation Reagent

When working with solid samples, such as animal meals, use grams as the unit. Assume that 1 g of solids is equal to 1 mL of Preparation Reagent. (Note that dilution has no units.)

*Example:*

1 g of meat meal + 9 mL of Preparation Reagent

$$D = 1:(1 + 9)$$

$$D = 1:10$$

### RULE #2

The final dilution in a serial dilution is the product of all the dilutions that preceded it.

In serial dilutions, a sample that has already been diluted at least once is used as the source for further dilution. See example below.

#### FIRST DILUTION:

Sample A:

1g of meat meal + 3 mL of Preparation Reagent

$$D = 1:(1 + 3)$$

$$D = 1:4$$

#### SECOND DILUTION:

100  $\mu$ L Sample A (prepared as shown above)

+ 1,400  $\mu$ L Preparation Reagent

$$D = 100:(100 + 1,400)$$

$$D = 100:1,500 \text{ or } 1:15$$

#### RESULTING FINAL DILUTION:

$$1:4 \text{ (first dilution)} \times 1:15 \text{ (second dilution)} = 1:60$$



# ADDITIONAL DILUTIONS QUICK CARD

## General Guidelines for Dilutions

When a sample result is greater than the value of the highest calibrator, the instrument will flag the results as "HI". The sample must be prepared at a higher dilution and retested. Unfortunately, there is no rule for determining the higher dilution when a sample flags "HI". Experience with the Saffest™ System and products tested will help the operator gain a feel for determining the higher dilution.

Below are examples of serial dilutions and suggested dispensing amounts.

### Starting with an initial dilution of 1:4

1. To make a 1:8 dilution from the initial 1:4 dilution prepare a 1:2 dilution.  
*Example: Aliquot 200 µL of sample to a glass test tube and add 200 µL of Preparation Reagent.*
2. To make a 1:32 dilution from the initial 1:4 dilution prepare a 1:8 dilution.  
*Example: Aliquot 100 µL of sample to a glass test tube and add 700 µL of Preparation Reagent.*
3. To make a 1:64 dilution from the initial 1:4 dilution prepare a 1:16 dilution.  
*Example: Aliquot 100 µL of sample to a glass test tube and add 1500 µL of Preparation Reagent.*
4. To make a 1:256 dilution from the initial 1:4 dilution prepare a 1:64 dilution as outlined above. Using the 1:64 diluted sample prepare a 1:4 dilution.  
*Example: Aliquot 200 µL of sample to a glass test tube and add 600 µL of Preparation Reagent.*

### Starting with an initial dilution of 1:10

1. To make a 1:20 dilution from the initial 1:10 dilution prepare a 1:2 dilution.  
*Example: Aliquot 200 µL of sample to a glass test tube and add 200 µL of Preparation Reagent.*
2. To make a 1:100 dilution from the initial 1:10 dilution prepare a 1:10 dilution.  
*Example: Aliquot 100 µL of sample to a glass test tube and add 900 µL of Preparation Reagent.*
3. To make a 1:500 dilution from the initial 1:10 dilution prepare a 1:100 dilution as outlined above. Using the 1:100 diluted sample prepare a 1:5 dilution.  
*Example: Aliquot 200 µL of sample to a glass test tube and add 800 µL of Preparation Reagent.*

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