Creating Linear and Serial Dilution Methods on an Agilent 7696A WorkBench

User's Guide

This user guide describes procedures for creating linear and serial dilutions for a four level calibration curve complete with internal standards. It can be used as a template for creating similar calibration curves with an Agilent 7696A WorkBench.

Linear dilutions: In this example, we start with a 2,000 ppb stock standard and make a series of four linear dilutions such that the resulting concentrations are: 1,000 ppb, 500 ppb, 250 ppb, and 125 ppb.

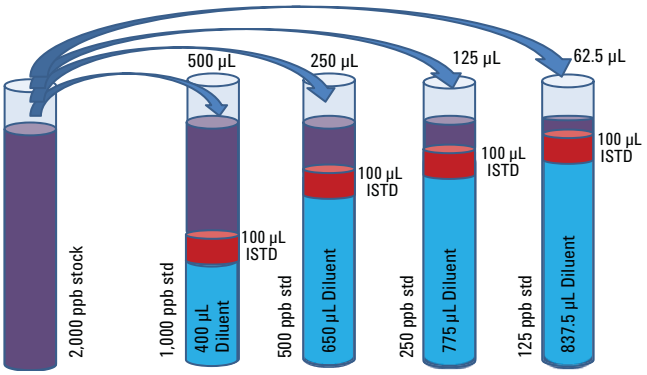

Figure 1. Linear dilutions.

Serial dilutions: In this example, we start with a 2,000 ppb stock standard and make a series of four serial dilutions such that the resulting concentrations are: 1,000 ppb, 500 ppb, 250 ppb, and 125 ppb.

Each of the four calibration standards have the same volume of internal standard added.

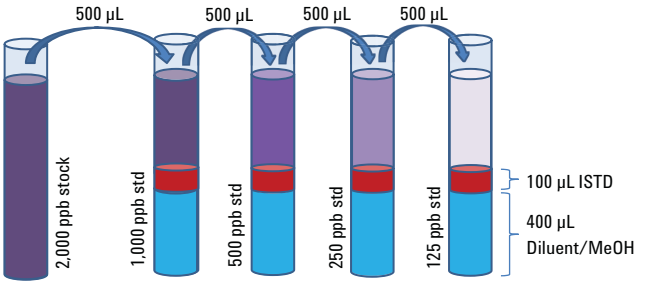

Figure 2. Serial dilutions.

Resource Layout Editor

Before building the method for WorkBench, the resources must be allocated using the Resource Layout (Figure 3).

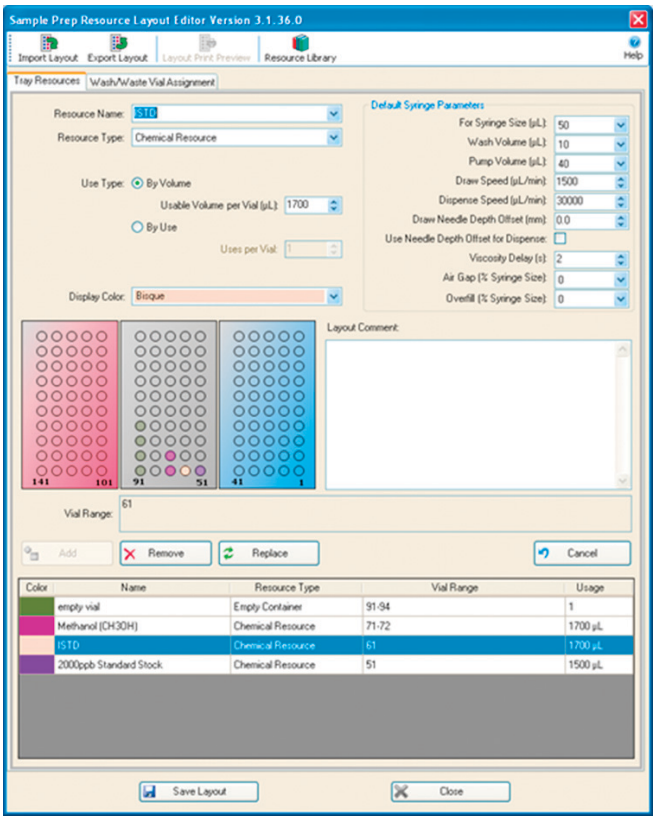

Figure 3. Resource layout.

- *Empty vials – calibration standards are made in these*
- *Methanol - diluent*
- *ISTD – internal standard*
- *2,000 ppb standard stock – starting stock solution*

Methanol, ISTD, and 2,000 ppb standard stock can be allocated by volume (the user selects the amount of volume available in the vial[s]) or by use (the user selects how many times the resource can be accessed). Most chemical resources are allocated as "by volume", however "by use" is helpful when the resource evaporates or degrades quickly after the vial has been pierced.

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Create Prep Method - Linear Dilution

Using the Resources allocated above the Agilent 7696A WorkBench method is created stepwise (shown below and in Figure 4).

1. Add 400 µL of methanol to an empty vial, which is renamed ‘1,000 ppb std’.
2. Add 100 µL of the resource ‘ISTD’ to the newly created vial ‘1,000 ppb std’.
3. Add 500 µL of the resource ‘2,000 ppb Standard Stock’ to ‘1,000 ppb std’.
4. Mix ‘1,000 ppb std’ (1,000 ppb std is now complete).
5. Add 650 µL of methanol to an ‘empty vial’ which is renamed ‘500 ppb std’.
6. Add 100 µL of the resource ‘ISTD’ to the newly created vial ‘500 ppb std’.
7. Add 250 µL of the resource ‘2,000 ppb Standard Stock’ to ‘500 ppb std’.
8. Mix ‘500 ppb std’ (500 ppb std is now complete).
9. Add 775 µL of methanol to an empty vial, which is renamed ‘250 ppb std’.
10. Add 100 µL of the resource ‘ISTD’ to the newly created vial ‘250 ppb std’.
11. Add 125 µL of the resource ‘2,000 ppb Standard Stock’ to ‘250 ppb std’.
12. Mix ‘250 ppb std’ (250 ppb std is now complete).
13. Add 830 µL of methanol to an empty vial, which is renamed ‘125 ppb std’.
14. Add 7.5 µL of methanol to the newly created vial '125 ppb std'.
15. Add 100 µL of the resource ‘ISTD’ to ‘125 ppb std’.
16. Add 62.5 µL of the resource ‘2,000 ppb Standard Stock’ to ‘125 ppb std’.
17. Mix ‘125 ppb std’ (125 ppb std is now complete).
18. Flag the vials created: 1,000 ppb std, 500 ppb std, 250 ppb std, and 125 ppb std as ‘result vials’.

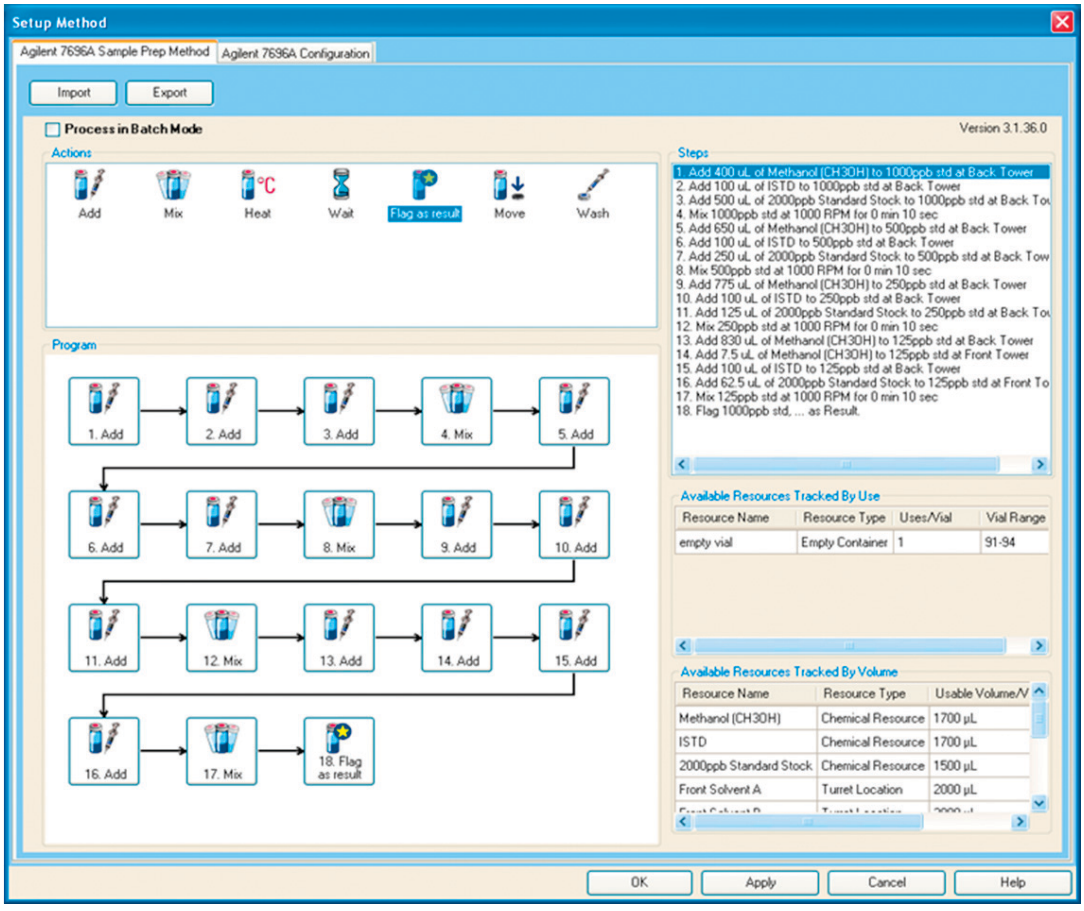

Figure 4. Create prep method, linear dilution.

Create Prep Method - Serial Dilution

Using the Resources allocated above the Agilent 7696A WorkBench method is again created stepwise (shown below and in Figure 5).

1. Add 400 µL of methanol to an empty vial, which is renamed '1,000 ppb std'.
2. Add 100 µL of the resource 'ISTD' to the newly created vial '1,000 ppb std'.
3. Add 500 µL of the resource '2,000 ppb Standard Stock' to '1,000 ppb std'.
4. Mix '1,000 ppb std' (1,000 ppb std is now complete).
5. Add 400 µL of methanol to an empty vial, which is renamed '500 ppb std'.
6. Add 100 µL of the resource 'ISTD' to the newly created vial '500 ppb std'.
7. Add 500 µL of the resource '1,000 ppb std' to '500 ppb std'.
8. Mix '500 ppb std' (500 ppb std is now complete).
9. Add 400 µL of methanol to an empty vial, which is renamed '250 ppb std'.
10. Add 100 µL of the resource 'ISTD' to the newly created vial '250 ppb std'.
11. Add 500 µL of the resource '500 ppb std' to '250 ppb std'.
12. Mix '250 ppb std' (250 ppb std is now complete).
13. Add 400 µL of methanol to an empty vial, which is renamed '125 ppb std'.
14. Add 100 µL of the resource 'ISTD' to the newly created vial '125 ppb std'.
15. Add 500 µL of the resource '250 ppb std' to '125 ppb std'.
16. Mix '125 ppb std' (125 ppb std is now complete).
17. Flag the vials created: 1,000 ppb std, 500 ppb std, 250 ppb std, and 125 ppb std as 'result vials'.

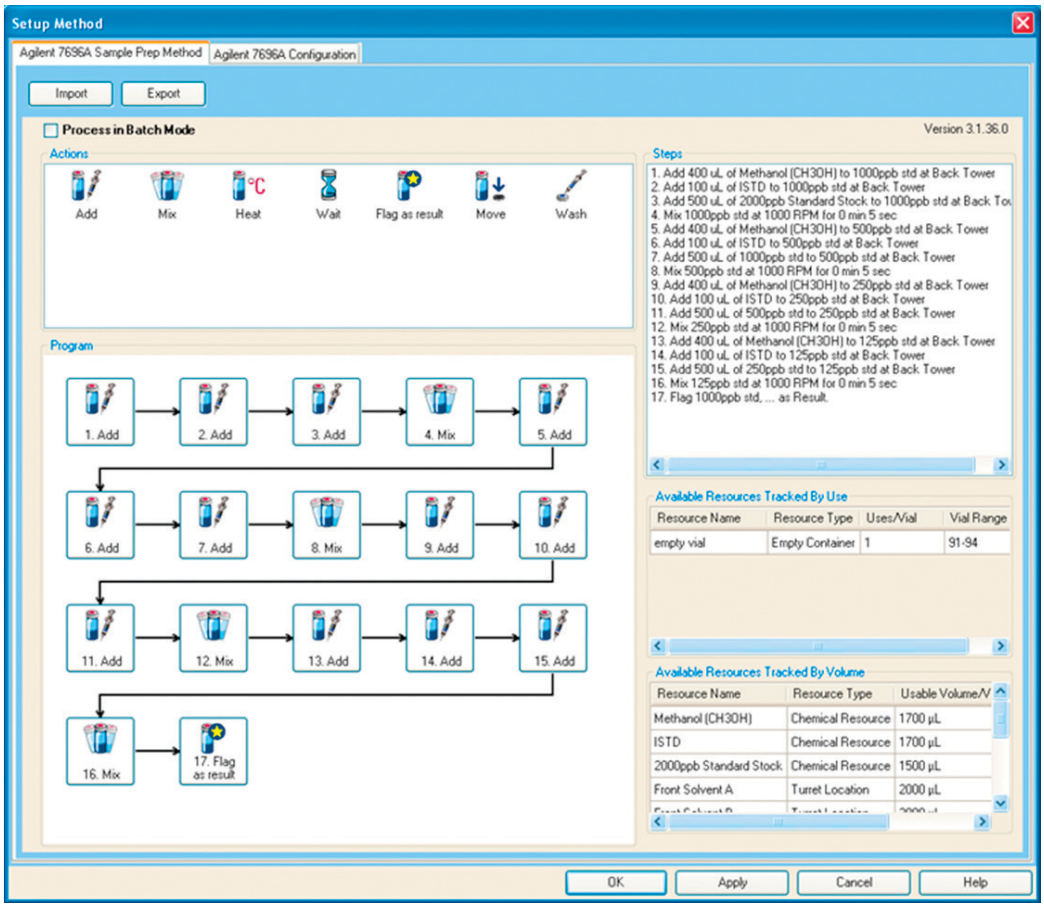

Figure 5. Create prep method, serial dilution.

Easy Sequence

To run the method created to generate the four calibration standards (Figure 6):

1. Select **Easy Sequence**.
2. Select your saved method.
3. Under **Starting Vial Location** enter any vial position not used in the Resource Layout (for this example we chose vial 1).
4. Under **Number of Samples** enter **1**.

You must enter values here even though there are no 'samples' when running a method to create calibration standards is used.

5. Under **Sample Name** enter a name (optional).
6. Select **Fill Samples** and start the sequence as you would normally.

This sequence will be added to your sequence queue.

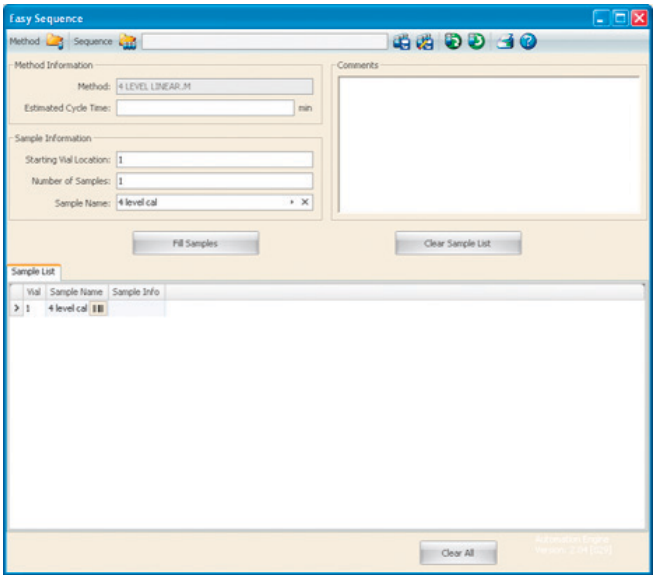

Figure 6. Easy sequence.

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