Label test tubes for Standards (Calibrators), Control, and Samples.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>Diluent/Zero</td>
</tr>
<tr>
<td>3, 4</td>
<td>Standard 1, 0.05 ppb</td>
</tr>
<tr>
<td>5, 6</td>
<td>Standard 2, 0.125 ppb</td>
</tr>
<tr>
<td>7, 8</td>
<td>Standard 3, 0.25 ppb</td>
</tr>
<tr>
<td>9, 10</td>
<td>Standard 4, 0.5 ppb</td>
</tr>
<tr>
<td>11</td>
<td>Sample 1</td>
</tr>
<tr>
<td>12</td>
<td>Sample 2</td>
</tr>
<tr>
<td>13</td>
<td>Sample 3</td>
</tr>
</tbody>
</table>

Add 500 μL of either Standards, Control or Samples down the inside wall of each test tube by aiming the pipet tip 1/4” to 1/2” below the tube rim without touching the rim or tube wall with the pipet tip; deliver liquid gently.

Add 500 μL of either Standards, Control or Samples down the inside wall of each test tube by aiming the pipet tip 1/4” to 1/2” below the tube rim without touching the rim or tube wall with the pipet tip; deliver liquid gently.

Add 250 μL of the Spinosyn Antibody Solution to the bottom of each tube by inserting the pipette tip all the way into the bottom of the tube without touching the side of the tubes.

React 15 minutes at room temperature (15° - 30°C).

Add 250 μL of Spinosyn Enzyme Conjugate down the inside wall of each tube by using the technique described in Box 2. Vortex or swirl for 5 to 10 seconds.

React 15 minutes at room temperature (15° - 30°C).

Add 4 mL of Washing Solution to each tube (alternatively flood the tubes completely with wash solution then invert to empty tubes). Using a smooth motion, invert tubes over a sink and pour out the tube contents: keep inverted and blot the test tube rims on several layers of paper toweling. Repeat this step 4 times for a total of five washes.

Add 500 μL of Color Reagent down the inside wall of each tube by using the technique described in Box 2.

React for 15 minutes at room Temperature (15° - 30°C). During this period, add 1 mL of Washing Solution into a clean tube for use as an instrument blank in Step 8.

Add 500 μL of Stopping Solution down the inside wall of each tube by using the technique previously described. Read results at 450 nm within 15 minutes after adding the Stopping Solution. Multiply results of samples by the appropriate dilution factor (if any).

[Safety Caution: Stopping Solution contains diluted sulfuric acid.]

For Ordering or Technical Assistance Contact:
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Web: www.abraxiskits.com

Spinosyn Tube Kit Part # 50020B, 40 Test
1. Add 500 μL of either Standards, Control or Samples to the bottom of each test tube.

2. Add 250 μL of Antibody Solution to each test tube.

3. Incubate for 15 minutes.

4. Add 250 μL of Spinosyn Enzyme Conjugate to each test tube.

   Vortex or swirl.

5. Incubate for 15 minutes.

6. Add 4 mL of Washing Solution (alternatively flood the tubes).

   Invert the tubes and blot.

   Repeat this step 4 times for a total of five washes.

7. Add 500 μL of Color Reagent down the inside wall of each test tube.

8. Incubate for 15 minutes.

   Prepare blank.

9. Add 500 μL of Stopping Solution to each test tube.

   Read OD 450