Sample spiking and recovery calculation for ELISA tests

1. **Experiment overview**
   2. Prepare homogenous matrix. The matrix should be blank – it should contain no analyte. If it is not possible to find negative matrix, use the matrix with the lowest analyte level possible.
   3. Weigh out two samples of the required weight (as stated in the procedure for the sample extraction from the ELISA kit manual); next, label one of them “negative” and the other one “positive”.
   4. Spike the “positive” sample with calculated volume of a standard solution to obtain a required spiking level. Mix the sample.
   5. Leave the samples to equilibrate for 30 min–1h.
   6. Analyze both “negative” and “positive” samples and calculate the results.
   7. Calculate the recovery.

2. **Calculation of spiking solution volume to obtain a sample with required level of analyte expressed in ppb (ng/g)**

The following calculation can be used if you know the level of analyte you want to use in your sample, for example you want to determine the method performance at a regulatory level. You will need a solution of the analyte for spiking typically at the concentration 50-100 times the spiking level. You will also need the information about sample weight that is used in the sample preparation method from the kit manual.

<table>
<thead>
<tr>
<th>Sample weight [g]</th>
<th>Required spiking level [ppb=ng/g]</th>
<th>Concentration of standard solution for spiking [ng/mL]</th>
</tr>
</thead>
<tbody>
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</table>

Volume of the standard solution to use for spiking can be calculated form the formula:

\[
Volume \text{ of standard solution} = \frac{sample \ weight \times spiking \ level}{concentration \ of \ standard}
\]

**Example**

You are using Total Aflatoxin ELISA to analyze food sample and you want to spike your sample at 2 ppb level. You have aflatoxin B₁ standard at a level of 1000 ng/mL available for spiking. Sample weight is given in the sample preparation procedure for food samples and it is 3 g.
**Sample weight [g]** | 3  
---|---  
**Required spiking level [ppb=ng/g]** | 2  
**Concentration of standard solution for spiking [ng/mL]** | 1000  
**Volume of standard to use [mL]** | ?

*Volume of standard solution* = \( \frac{3 \, \text{g} \times 2 \, \frac{\text{ng}}{\text{g}}}{1000 \, \frac{\text{ng}}{\text{mL}}} = 0.006 \, \text{mL} = 6 \, \mu\text{L} \)

We do not recommend using spiking volumes less than 20-25 µL. The spiking volume should be also less than 5% of the total volume of extraction solvent used. 50-100 µL spiking volumes are recommend. For the given example it is not recommended to use 6 µL for spiking, but it is better to dilute the available standard to 100 ng/mL and perform the calculations again. In order to dilute 1000 ng/mL standard to 100 ng/mL, you need to dilute it 10 times. For example take 100 µL of 1000 ng/mL standard and add it to 900 µL of solvent to obtain 100 ng/mL solution.

**Sample weight [g]** | 3  
---|---  
**Required spiking level [ppb=ng/g]** | 2  
**Concentration of standard solution for spiking [ng/mL]** | 100  
**Volume of standard solution for spiking [mL]** | ?

*Volume of standard solution for spiking* = \( \frac{3 \, \text{g} \times 2 \, \frac{\text{ng}}{\text{g}}}{100 \, \frac{\text{ng}}{\text{mL}}} = 0.06 \, \text{mL} = 60 \, \mu\text{L} \)

If 60 µL of 100 ng/mL aflatoxin B1 solution is added to the sample then the sample containing 2 ppb of aflatoxin B1 is obtained.

3. **Calculation of spiking solution volume to obtain a sample with required level of analyte expressed in ng/mL as read from the ELISA standard curve**

If you are not sure what level you should spike at but you want to determine method recovery, it is recommended to check the example calibration curve in the kit manual and select the concentration in the middle of the standard curve expressed in ng/mL. This is the level that will give you the most accurate results. You can also select any other level: for example 80% Bmax to check method performance close to LOD level or Bmax 20% to check method performance at higher concentration level. Read the approximate analyte concentration from the standard curve corresponding to the selected Bmax.

You will need a solution of the analyte for spiking typically at the concentration 50-100 times the spiking level. You will also need to find the information about sample weight that is used for the sample preparation and dilution or concentration factor for the method. This information is given in each kit.
If the interpretation of results section of the manual says “the results have to be multiplied by a factor X to obtain analyte content in a sample” – then X is a dilution factor for the method. If the instruction says “the results have to be divided by a factor of X…” then X is a concentration factor for the method.

<table>
<thead>
<tr>
<th>Sample weight [g]</th>
<th>3</th>
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<tbody>
<tr>
<td>Dilution factor or concentration factor</td>
<td>16</td>
</tr>
<tr>
<td>Required level read from ELISA standard curve [ng/mL]</td>
<td>0.03</td>
</tr>
<tr>
<td>Concentration of standard solution for spiking [ng/mL]</td>
<td>10</td>
</tr>
<tr>
<td>Volume of standard solution for spiking [mL]</td>
<td>?</td>
</tr>
</tbody>
</table>

For method with dilution factor:

\[ \text{Spiking level} = \text{level read from ELISA standard curve} \times \text{dilution factor} \]

For method with concentration factor:

\[ \text{Spiking level} = \frac{\text{level read from ELISA standard curve}}{\text{concentration factor}} \]

The volume of the standard solution to use can be calculated from the formula:

\[ \text{Volume of standard solution} = \frac{\text{sample weight} \times \text{spiking level}}{\text{concentration of standard}} \]

Example

You are using Total Aflatoxin ELISA to analyze food sample. You want to analyze a sample that will result in 50% Bmax. From the standard curve included in the kit manual, 50% Bmax corresponds to approximately 0.03 ng/mL of aflatoxin B₁. You have aflatoxin B₁ standard at a level of 10 ng/mL available for spiking. Sample weight is given in the extraction procedure for food samples and it is 3 g. The interpretation of results section for food extraction procedure states that the result read from the calibration curve has to be multiplied by a factor of 16 so 16 is the dilution factor for the sample preparation method.

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<tr>
<td>Volume of standard solution for spiking [mL]</td>
<td>?</td>
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Spiking level \( = 0.03 \times 16 = 0.48 \frac{ng}{g} \)

Volume of standard solution \( = \frac{3 \times 0.48 \frac{ng}{g}}{10 \frac{ng}{mL}} = 0.144 mL = 144 \mu L \)

4. Calculation of recovery

After analysis, calculate the concentration of the analyte in a “negative” and spiked sample.

<table>
<thead>
<tr>
<th>Spiking concentration [ng/g]</th>
<th>Concentration measured by ELISA in spiked sample [ng/g]</th>
<th>Concentration measured by ELISA in negative samples [ng/g]</th>
<th>Recovery [%]</th>
</tr>
</thead>
</table>

Calculate the recovery from the formula:

\[
Recovery = \frac{conc. \text{ measured in spiked sample} - conc. \text{ measured in negative sample}}{spiking \text{ concentration}} \times 100\%
\]

Example

Total aflatoxin ELISA was used to analyze a negative sample and a sample spiked at 2 ppb. The measured concentration for the spiked sample in ng/mL read from the standard curve was 0.14 ng/mL what should be multiplied by a factor of 16 to obtain aflatoxin B₁ content in the sample – 2.24 ng/g. The negative sample gave a reading of 0.02 ng/mL of aflatoxin B₁ which can be converted to 0.32 ng/g in a sample.

<table>
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<tr>
<th>Sample preparation dilution factor</th>
<th>Spiking concentration [ppb=ng/g]</th>
<th>Concentration measured by ELISA in spiked sample [ng/mL]</th>
<th>Concentration measured by ELISA in negative sample [ng/mL]</th>
<th>Concentration measured by ELISA in spiked sample [ng/g]</th>
<th>Concentration measured by ELISA in negative sample [ng/g]</th>
<th>Recovery [%]</th>
</tr>
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<tbody>
<tr>
<td>16</td>
<td>2</td>
<td>0.14</td>
<td>0.02</td>
<td>2.24</td>
<td>0.32</td>
<td></td>
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Then recovery can be then calculated as follows:

\[
Recovery = \frac{2.24 \frac{ng}{g} - 0.32 \frac{ng}{g}}{2 \frac{ng}{g}} \times 100\% = 96\%
\]