

EXTRACTION EFFICIENCY PROTOCOL

Extraction efficiency is a calculation that is performed in order to determine how well you are able to recover a targeted molecule post-extraction of a biological sample or solution. Extraction efficiency is calculated as the net amount of spiked material that is recovered post-extraction, divided by the amount of spiked material detected in the assay buffer. This number is usually expressed as a percentage. Required for this extraction efficiency determination is a single representative sample which will need to be split into two equal aliquots as well as a non-extracted Assay Buffer Control.

1. Pipet an equal volume of a sample into fresh tubes labeled as “+spike” and “-spike.”
2. Pipet the same volume of Assay Buffer into a fresh tube labeled as “Assay Buffer Control.”
3. Spike the “+ spike” tube and the “Assay Buffer Control” tube with a known concentration of the targeted molecule using the standard provided in the kit. Generally speaking it is best to pick a concentration that will fall around the middle of the curve unless you plan to account for dilution/concentration of the sample post-extraction in which case a higher/lower concentration should be chosen. Be sure to add the same volume of assay buffer to the “-spike” tube so that it will be at the same volume as the “+spike” and the “Assay Buffer Control” tube.
4. Cap and store the “Assay Buffer Control” containing the spiked standard at or below -20°C. Note: This control will not be put through the extraction protocol so will not be needed until the assay is to be run.
5. Extract the “+spike” sample and the “-spike” sample according to the extraction procedure.
6. Dry down completely and reconstitute the samples as directed. Note: If you reconstitute in the starting volume of the sample there will be no need to adjust for a dilution/concentration factor of the original sample. At a minimum, you will need to reconstitute in enough volume to run the assay.
7. Thaw the “Assay Buffer Control” tube. Run the reconstituted samples (+spike and -spike) and “Assay Buffer Control” in the assay with a standard curve diluted in Assay Buffer.
8. Calculate the concentration of the “+spike” sample and the “-spiked” samples as well as the “Assay Buffer Control.” Note: If you dilute or concentrate the sample when reconstituting then the appropriate dilution or concentration factor will need to be taken into account when calculating the target molecule concentration.

9. Calculate the net spiked standard that was detected after the extraction by subtracting the “-spike” extract concentration from the “+spike” extract concentration. Doing this will remove the endogenous level detected in the sample leaving only the recovered spike concentration.

$$\text{Net Spike Concentration} = (\text{“+spike” concentration}) - (\text{“-spike” concentration})$$

10. The recovered spike concentration is divided by the “Assay Buffer Control” concentration and multiplied by 100 to give the extraction efficiency percentage.

$$\% \text{ Extraction Efficiency} = (\text{Net Spike Concentration} / \text{Assay Buffer Control Concentration}) * 100$$

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