

Protocol for the reconstitution of lyophilised in house reference material

Reconstitution of a lyophilised sample is realised by adding the amount of water lost during lyophilisation. To guarantee uniform sample reconstitution and to avoid any potential inhomogeneity, it is recommended to reconstitute the total amount of lyophilised sample. In any case, the amount of lyophilised sample to be reconstituted shall not be smaller than the amount used in the homogeneity study, as indicated in the data sheet. Frequent temperature changes and opening of the sample vessels should be avoided. The vessels should be at room temperature before reconstitution, as the samples are hygroscopic.

The recommended method for the reconstitution of lyophilised samples is:

- Shake the sample by hand or agitate using a vortex-mixer or an overhead shaker
- Weigh out an adequate amount of lyophilised material, preferably the total amount
- Add respective amount of water as ultra-pure water and vortex-mix
- If vortex-mixing does not yield a visually uniform sample, treat the sample in an ultrasonic bath or homogenise using an overhead shaker for at least 15 min at ambient temperature.
- Ideally, the reconstituted sample is stored at +4 °C over night.
- Before analysis the reconstituted sample should be homogenised using a vortex-mixer, an overhead-shaker or ultrasonication.

The amount of ultra-pure water m_{water} required for reconstitution of a lyophilised sample is calculated from the dry weight (DW in %) given in the sample data sheet and the amount of lyophilised sample to be reconstituted m_{lyo} :

$$m_{water} = \frac{m_{lyo}}{DW} \cdot 100 - m_{lyo}$$

Example:

$$DW = 29.3 \% ; m_{lyo} = 2.00 \text{ g}$$

$$m_{water} = \frac{m_{lyo}}{DW} \cdot 100 - m_{lyo} = \frac{2.00 \text{ g}}{29.3 \%} \cdot 100 \% - 2.00 \text{ g}$$

$$m_{water} = 4.83 \text{ g}$$

Aliquots of the reconstituted sample should be stored at $(-20 \pm 2) ^\circ\text{C}$. Please refer to the data sheet or the FIS-VL for information on sample stability. Long-term storage of reconstituted samples is generally not recommended.