

## SOP for sample stability test

1. To define the sample, or biomarker, stability, perform the following steps for three independent samples, preferably with different concentrations of the measurand (low, medium, high). For a full validation we recommend to use all variables indicated below, however, dependent on the purpose of the stability testing some (in-between) steps may be left out.
2. Divide the sample into nineteen aliquots with equal sample volume.

*NB: It is important that every aliquot contains the same sample volume and to use the same kind of reaction vials, since unequal sample volumes may affect the concentration of the measurand due to adsorption or evaporation.*

### - Freeze-thaw stability -

3. Place aliquots #1-6 at  $-80^{\circ}\text{C}$ .
4. Thaw aliquots #2-6 and store again at  $-80^{\circ}\text{C}$ .

*Note: Thaw for 2 hours at room temperature and next store the sample at least 12 h at  $-80^{\circ}\text{C}$  for each freeze/thaw cycle.*

5. Thaw aliquots #3-6 and store again at  $-80^{\circ}\text{C}$  (see fig.2).
6. Thaw aliquots #4-6 and store again at  $-80^{\circ}\text{C}$ .
7. Thaw aliquot #5-6 and store again at  $-80^{\circ}\text{C}$ .
8. Thaw aliquot #5-6 and store again at  $-80^{\circ}\text{C}$ .
9. Thaw aliquot #6 and store again at  $-80^{\circ}\text{C}$ .
10. Thaw aliquot #6 and store again at  $-80^{\circ}\text{C}$ .

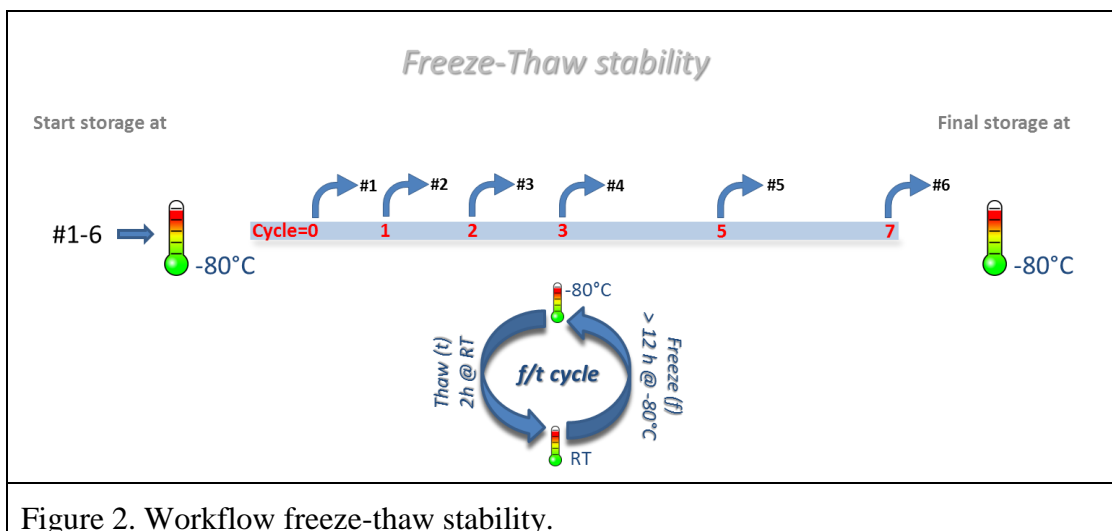


Figure 2. Workflow freeze-thaw stability.

**- Storage stability -**

- At time point 0, store aliquots #7-12 at room temperature and another six aliquots #13-18 at 4°C.
- At time points  $t=1h$ ,  $t=2h$ ,  $t=4h$ ,  $t=24h$ ,  $t=72h$ ,  $t=168h$ , transfer one sample stored at each temperature, RT and 4°C, to -80°C (see fig. 1).

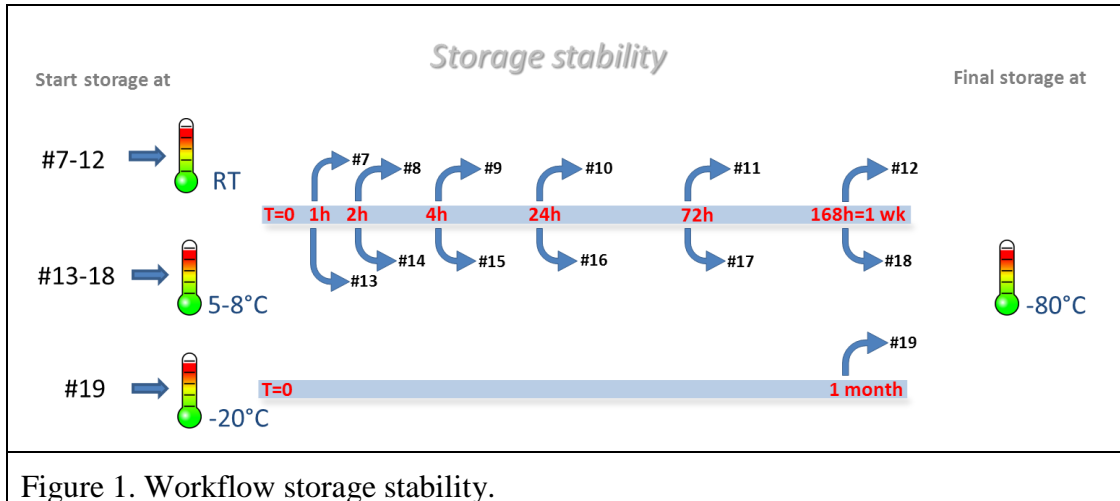


Figure 1. Workflow storage stability.

- Store aliquot #19 at -20°C during one month before transfer to -80°C.
- Thaw all aliquots for a given sample simultaneously and analyse them in the same run (in duplicates for standard ELISA assays).

*Note: All samples should be analysed in a randomised order using the same lot.*

**- Stability reporting -**

- Insert raw data of aliquots #1-19 (replicates of observed concentrations) in the Excel file STABCALC. The file calculates the mean value, standard deviation (SD), and coefficient of variation (%CV) for both the observed concentration and normalized concentration.

*Note: The standard deviation for the storage stability and the freeze-thaw stability should be within pre-defined acceptance criteria.*