

Sample collection and handling with Merckodia Glucagon ELISAs

In the Merckodia Glucagon ELISA both serum, EDTA plasma and cell culture samples can be used. However, glucagon will be sensitive to storage conditions and freeze/thaw cycles. This technical note shows results from sample stability studies for glucagon performed at Merckodia.

Summary

- For details about sample collection, please see pages 13-14.
- It is recommended to keep samples on ice when thawing them and preparing the assay. Return to freezer as soon as possible.
- Avoid storing samples at room temperature or 2-8 °C for long periods of time (Figure 1 and 11).
- Store samples at -80 °C (Figures 2 and 7).
- Avoid repeated freeze/thaw cycles (Figures 3, 5 and 12).
- The addition of aprotinin to samples will not improve glucagon stability (Figure 4).
- For studies in which very low levels of glucagon need to be detected, it is beneficial to stabilize plasma, e.g., by using tubes such as BD™ (Becton Dickinson) P800 for sample collection (art nr 366420/366421), since this will prevent the degradation of glucagon (Figure 6).
- Dilution in Calibrator 0 is recommended for all cell culture medium samples (Figure 10).
- If samples are to be stored, it is recommended to store as serum or plasma rather than as whole blood (Figure 9).
- Sample extraction does not improve glucagon yield (Figure 13).

Table of Contents

Serum and EDTA plasma samples	3
Short-term stability	3
Long-term stability	3
Freeze/thaw stability for serum and EDTA plasma	3
EDTA plasma with addition of aprotinin	5
Short-term stability	5
Freeze/thaw stability for EDTA plasma with the addition of aprotinin	5
EDTA plasma in BD™ P800 tubes	6
Short-term stability	6
Long-term stability	6
Freeze/thaw stability for EDTA plasma in BD™ P800 tubes	7
Whole blood samples	9
Short-term stability	9
Cell culture media samples	10
Dilutions	10
Freeze-thaw	11
Sample extraction	12
Recommendations for collecting, processing and storing samples	13
Serum samples	13
EDTA plasma samples	13
BD™ P800 plasma samples	14
References	14

Serum and EDTA plasma samples

Short-term stability

At 2-8 °C glucagon in serum or EDTA plasma is stable for at least 6 hours (Figure 1). For longer duration of storage, the degree of degradation is sample specific (i.e., storage at 2-8 °C for 24 hours can significantly affect glucagon concentrations for individual samples).

After 6 hours at room temperature glucagon concentrations in serum and EDTA plasma were found to be significantly reduced (Figure 1). This is consistent with *Wewer Albrechtsen et al. (2015)*, who reported that glucagon seemed relatively stable at RT for 1 hour but after 3 hours the recoveries dropped significantly.

Long-term stability

Glucagon in EDTA plasma samples was stable at both -20 °C and -80 °C for up to 12 months, but the average recovery for samples stored at -20 °C after 12 months is outside the acceptance criteria of $100 \pm 20\%$. Further studies are needed to verify glucagon stability in EDTA plasma at -20 °C since our findings do not match results reported by *Wewer Albrechtsen et al. (2015)*.

Freeze/thaw stability for serum and EDTA plasma

The Mercodia in-house study shows that serum and EDTA plasma should not be exposed to multiple freeze/thaw cycles since glucagon concentrations can decrease in the samples due to degradation (Figure 3)

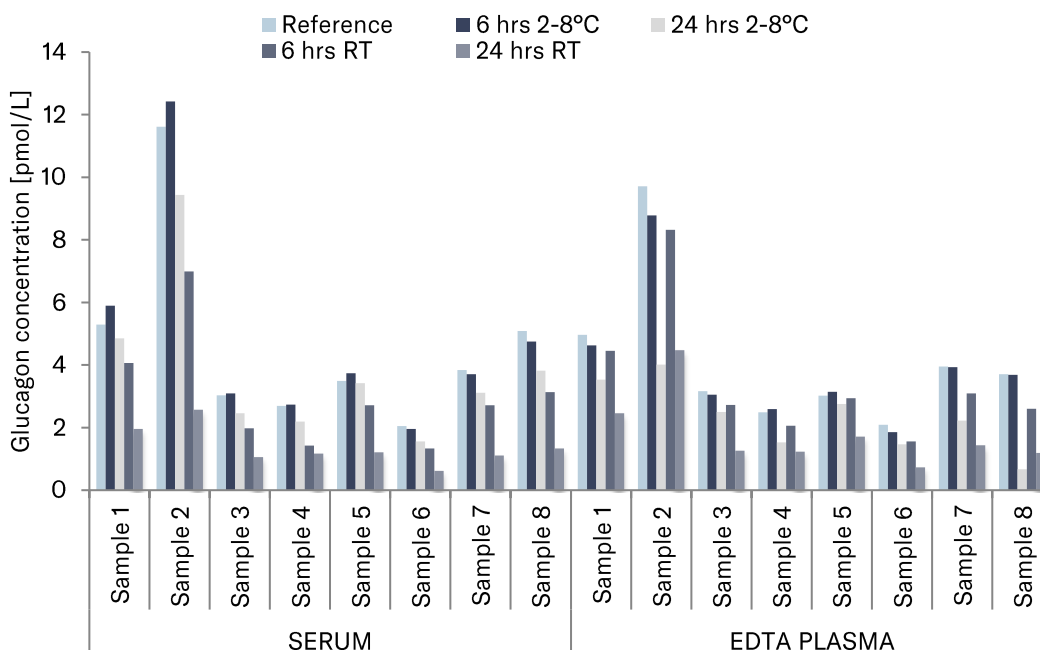


Figure 1. Short-term sample stability at different storage conditions for both serum and EDTA plasma samples.

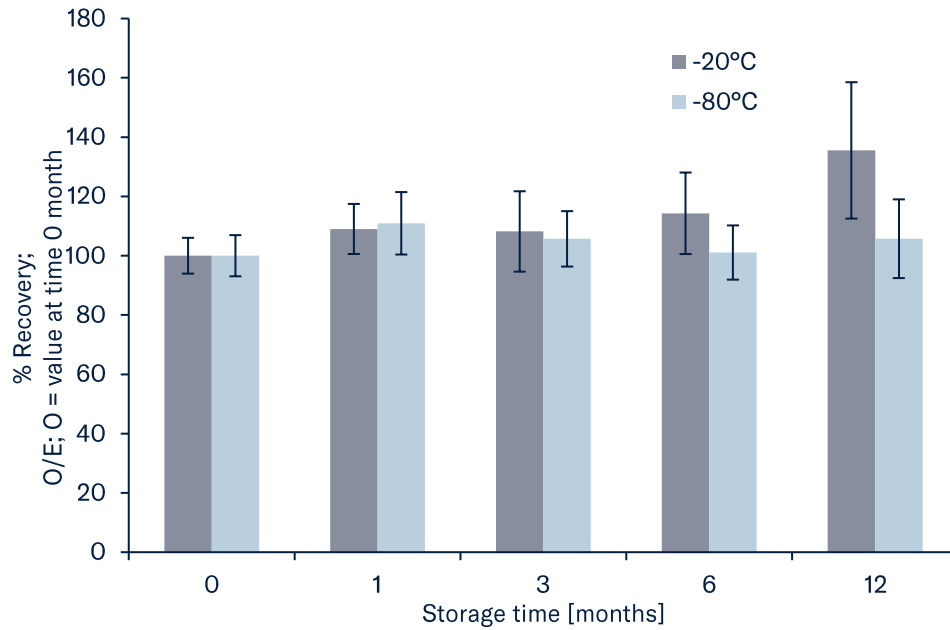


Figure 2. Long-term stability with average recoveries from 12 EDTA samples stored at -20 °C or -80 °C.

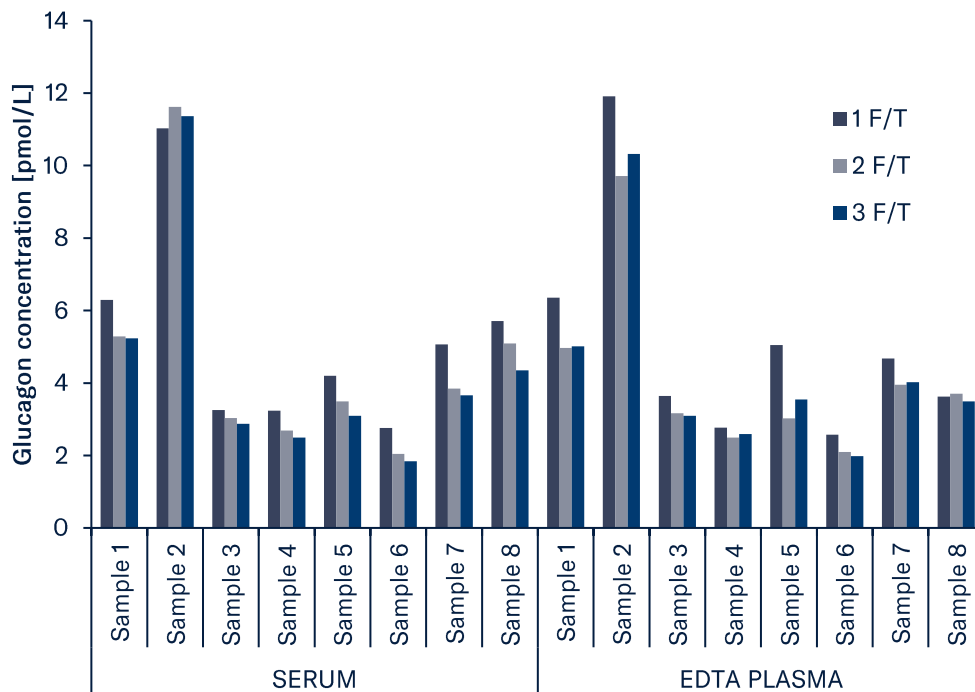


Figure 3. Sample stability after freeze/thaw (F/T) cycles for both serum and plasma samples.

EDTA plasma with addition of aprotinin

Short-term stability

Addition of aprotinin to EDTA plasma samples will not improve sample stability (Figure 4). Samples will still be sensitive to storage conditions. Therefore, Merckodia strongly suggests that samples should not be kept at room temperature. Storing samples for 6 hours at 2-8 °C is acceptable but for long-term storage the recommended temperature is -80 °C.

Freeze/thaw stability for EDTA plasma with the addition of aprotinin

Addition of aprotinin to EDTA plasma samples does not seem to change the effect of freeze/thaw cycles on glucagon recovery. Samples should not be exposed to multiple freeze/thaw cycles since glucagon concentrations can decrease in the samples due to degradation (Figure 5).

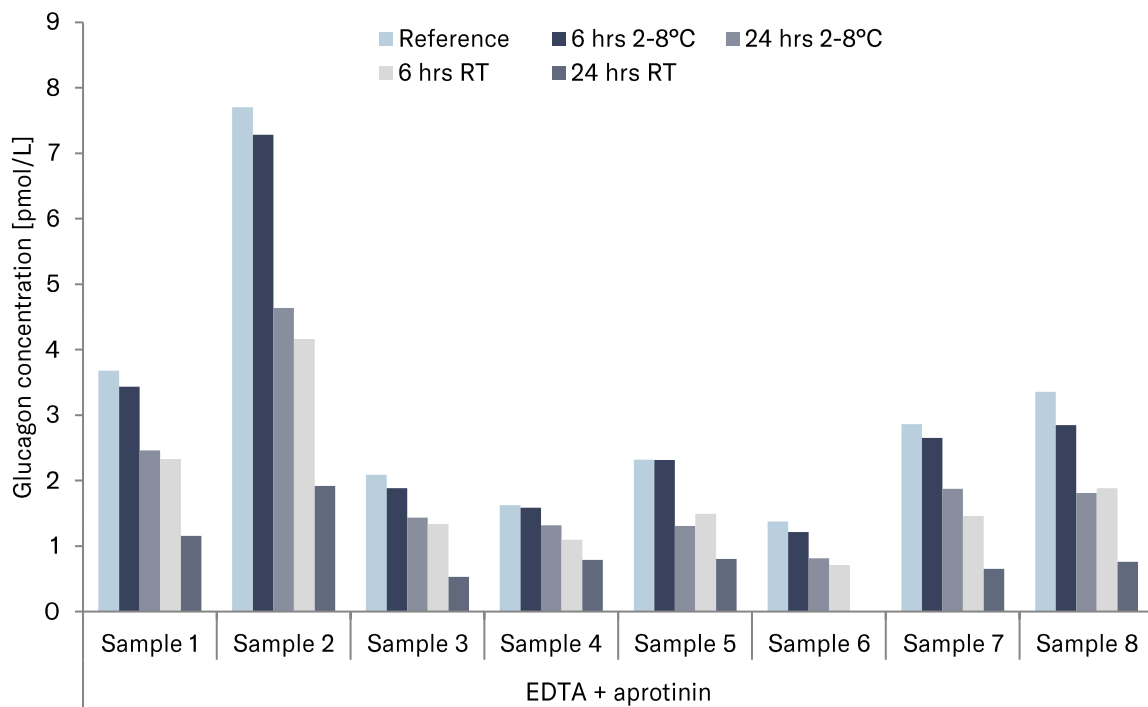


Figure 4. Short-term sample stability at different storage conditions for EDTA plasma samples with addition of aprotinin.

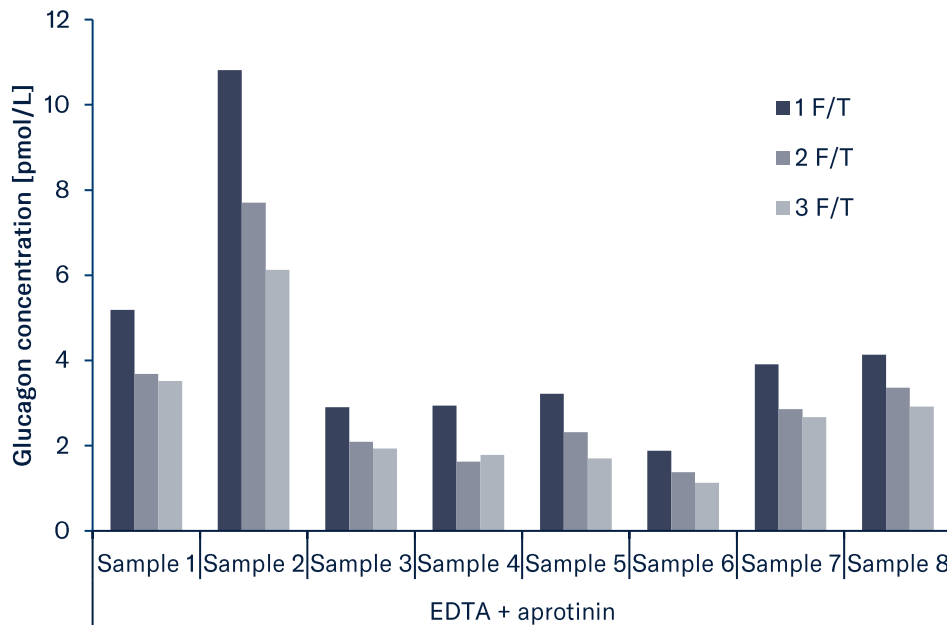


Figure 5. Sample stability after freeze/thaw (F/T) cycles for EDTA+aprotinin samples.

EDTA plasma in BD™ P800 tubes

Short-term stability

Collecting samples in Becton Dickinson (BD™) P800 tubes containing lyophilized protease (endonuclease) inhibitors and DPP-IV inhibitors will yield somewhat higher glucagon values than serum or EDTA plasma that is not collected in BD P800 tubes. This is most likely due to nonspecific protease inhibition since glucagon is an inefficient substrate for DPP-IV (Zhu *et al.*, 2003).

Samples from P800 tubes show acceptable stability for up to 24 hours at room temperature or 2-8 °C (Figure 6). However, sample specific differences cannot be excluded. Mercodia recommends storing samples at -80°C.

Long-term stability

P800 plasma samples from 130 apparently healthy individuals were analyzed after sample collection, and after storage at -80 °C for 13 and 27 months. The results show no significant difference with storage time (for up to 27 months of storage). This was also confirmed in a smaller study shown in Figure 7. Glucagon in P800 plasma samples are stable for at least 27 months when stored in -80 °C and for at least 12 months when stored in -20°C.

Freeze/thaw stability for EDTA plasma in BD™ P800 tubes

In this study, up to 3 freeze/thaw cycles are acceptable for glucagon measurements in P800 EDTA plasma if samples are stored at -80 °C and

kept on ice when thawed (Figure 8). Since sample specific differences cannot be excluded, Merckodia recommends avoiding freeze/thaw cycles

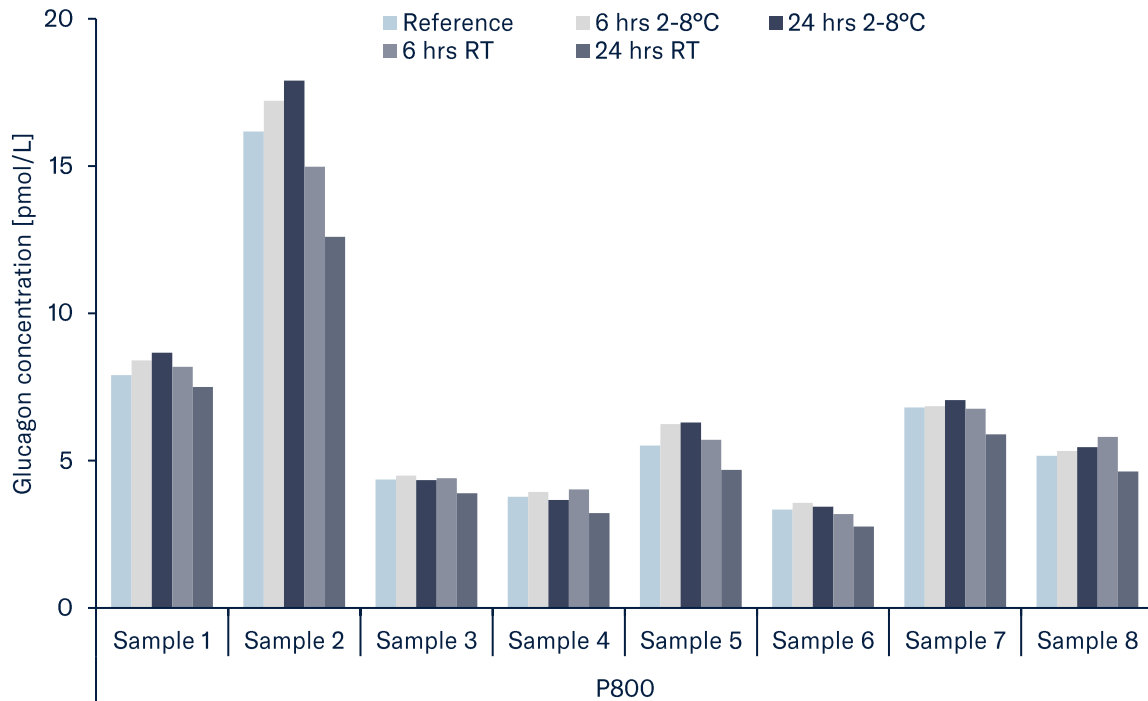


Figure 6. Short-term sample stability at different storage conditions for EDTA plasma in P800 tubes.

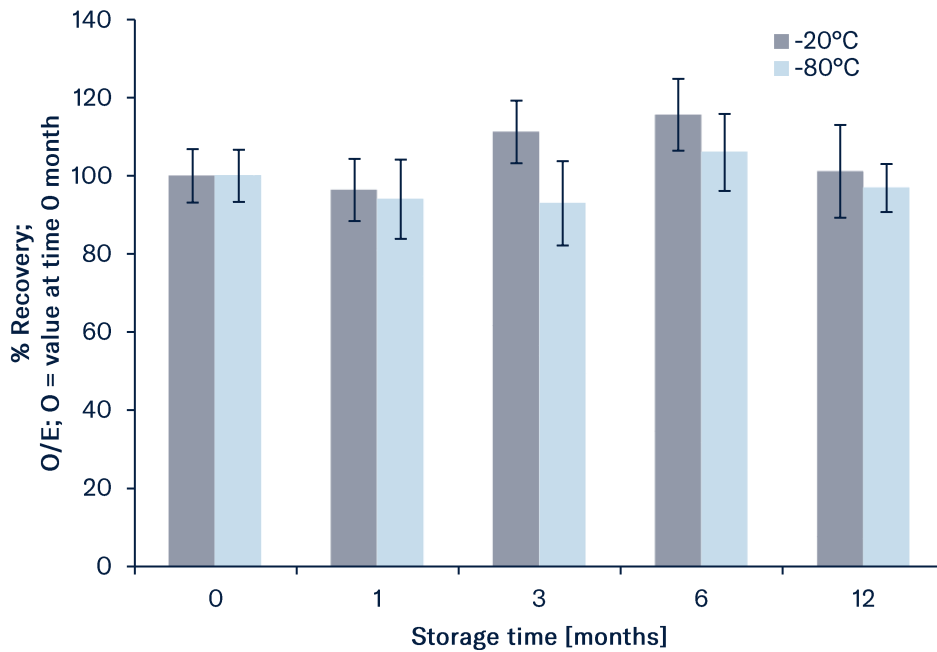


Figure 7. Long-term stability with average recoveries from 12 P800 plasma samples stored at -20 °C or -80 °C.

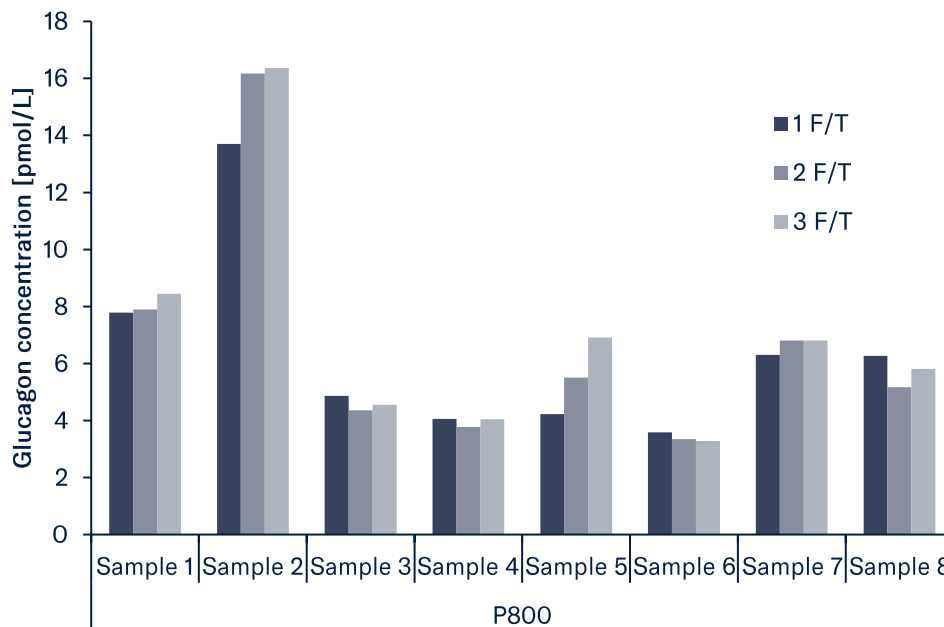


Figure 8. Sample stability after freeze/thaw (F/T) cycles for EDTA plasma in P800 tubes.

Whole blood samples

Short-term stability

Whole blood samples stored at 2-8 °C for 24 hours before preparation of plasma will show a decrease in glucagon levels for most samples

(Figure 9). Avoid storing samples as whole blood for longer periods of time.

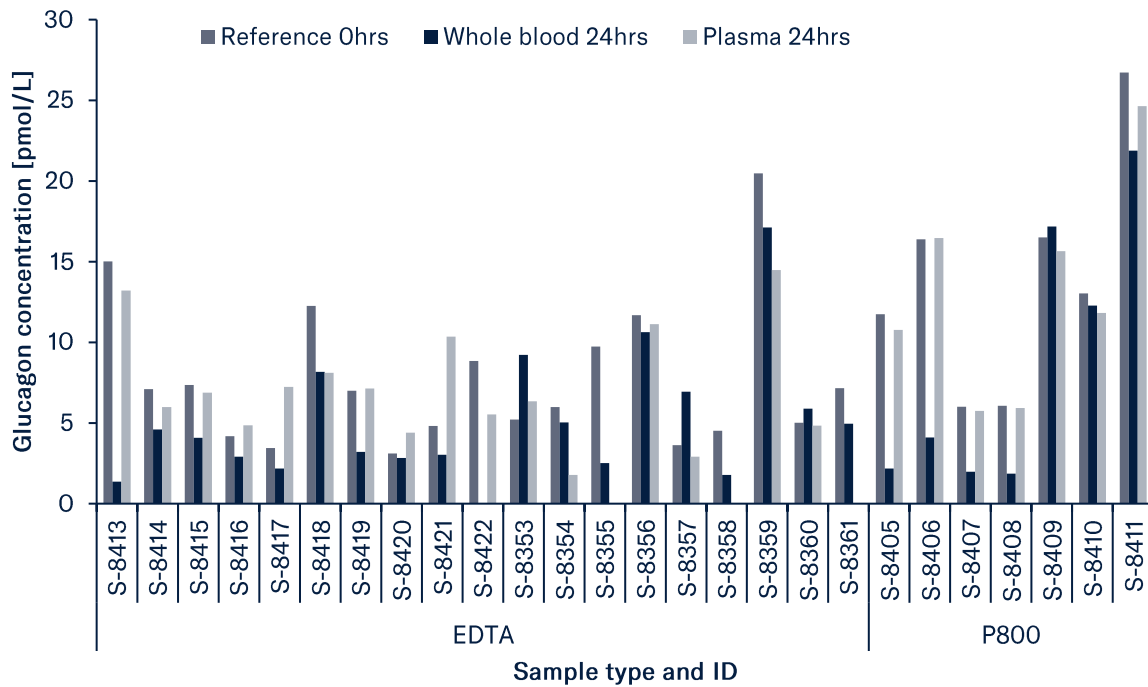


Figure 9. Samples stored as whole blood for 24 hours at 2-8 °C before preparing plasma (whole blood 24hrs) were compared to plasma samples prepared and analyzed immediately (reference 0hrs) or stored for 24 hours at 2-8 °C (plasma 24hrs) before being analyzed.

Cell culture medium samples

Dilutions

The measurement range for the Mercodia Glucagon ELISA is 1.5 – 130 pmol/L. In general, glucagon concentration in cell medium samples could be close to the upper limit of the assay range and, therefore, it is recommended that all cell culture medium samples are diluted with a

factor 2 (or more depending on the study design) to avoid reruns, Figure 10. Also, to avoid matrix effects, a factor 2 dilution or more is recommended. Calibrator 0 should be used as dilution buffer.

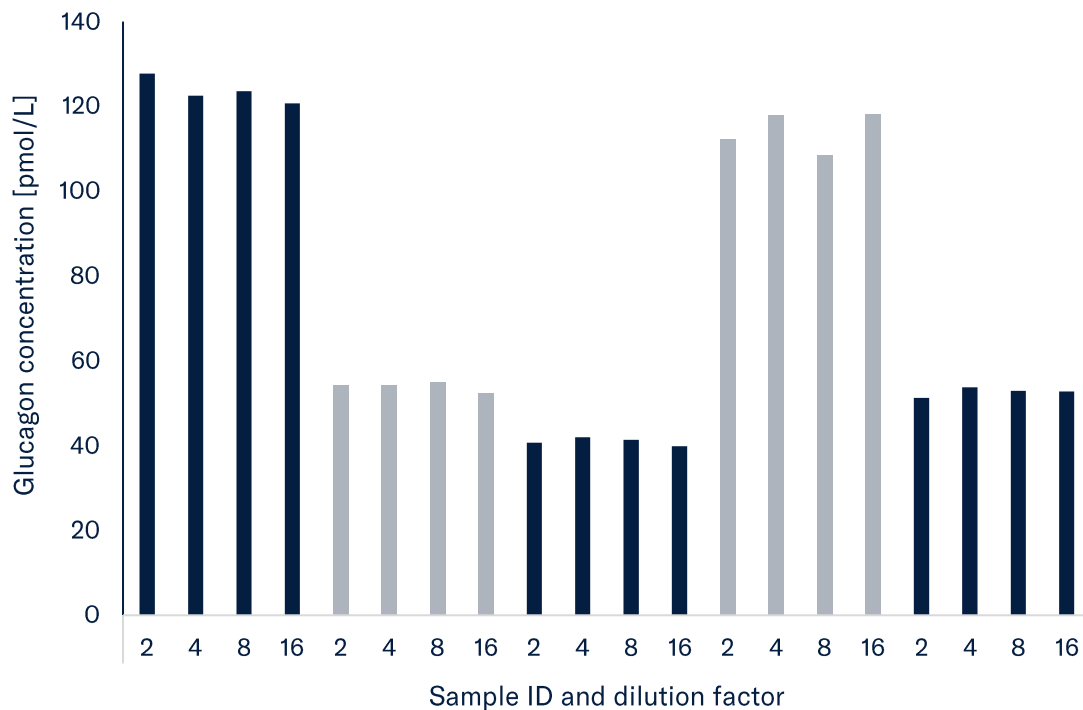


Figure 10. Cell culture medium samples diluted in Calibrator 0. Dilution factors 1, 2, 4, 8 and 16 were used to evaluate parallelism and minimum required dilution (MRD) for the sample type.

Short-term

With limited data on cell culture samples stored in room temperature, the recommendation is to keep samples on ice or in the cold room for shorter periods of time.

Avoid handling samples in room temperature, Figure 11.

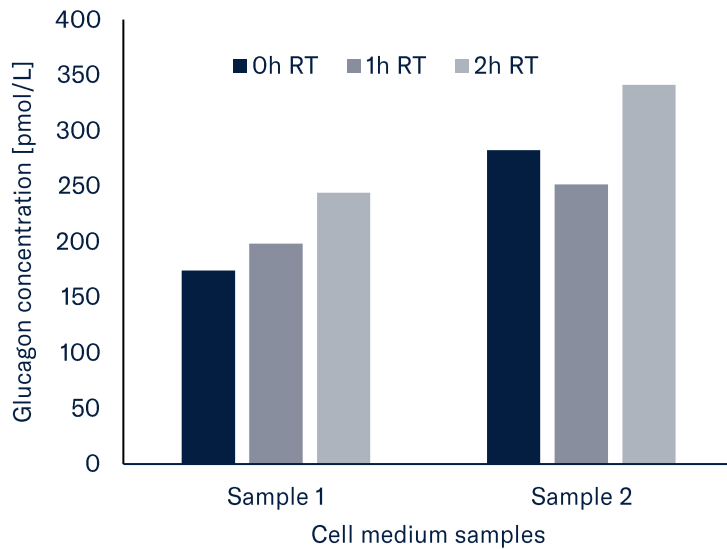


Figure 11. Short-term sample stability at room temperature for cell culture media samples.

Freeze-thaw

In this study, up to 3 freeze/thaw cycles are acceptable for glucagon measurements in cell culture medium samples if samples are kept on ice when thawed (Figure 12).

Since sample specific differences cannot be excluded, Mercodia recommends avoiding freeze/thaw cycles

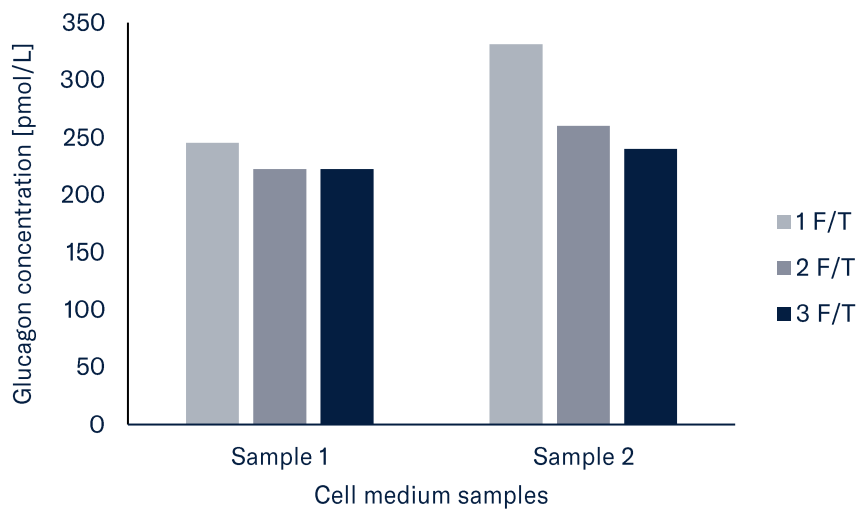


Figure 12. Sample stability after freeze/thaw (F/T) cycles for cell medium samples.

Sample extraction

Sample extraction does not improve glucagon yield in EDTA plasma (Figure 13).

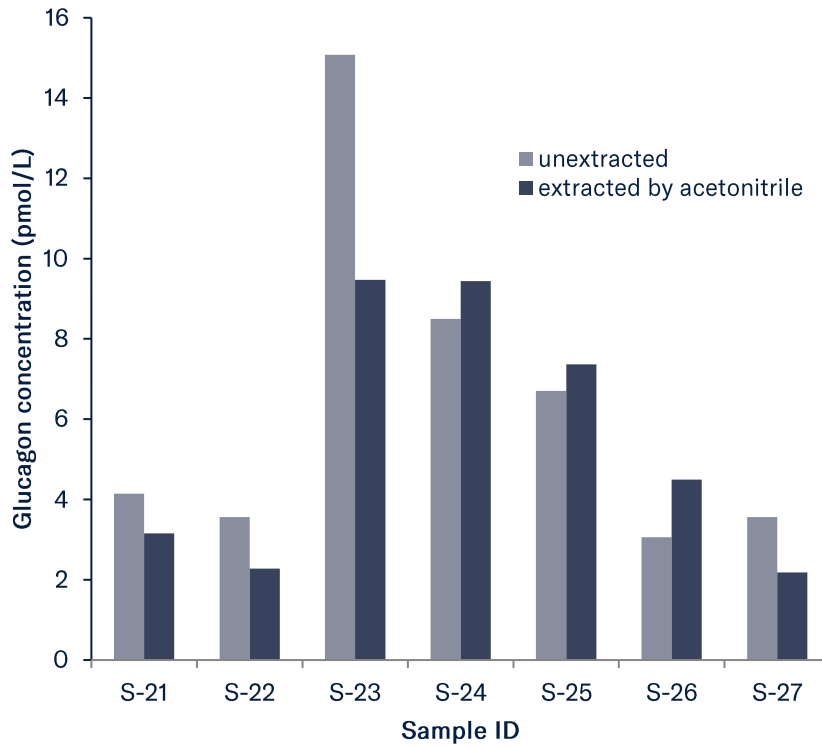


Figure 13. Glucagon concentrations in unextracted samples versus samples extracted by acetonitrile.

Recommendations for collecting, processing, and storing samples

The Project Manager or study protocol will dictate the venipuncture site and technique to be used. For additional details about sample processing, please refer to the instructions given by the tube manufacturer.

Serum samples

1. Collect blood by venipuncture into serum tubes.
2. Upon collection, mix the contents gently (without shaking) by inverting the tube.
3. Store the tube in a vertical position at room temperature to allow the blood to clot.
4. Collect the serum immediately after clotting by centrifugation.
5. Use a pipette to carefully transfer serum into appropriate vials.
6. Avoid storing samples at 2-8 °C for longer than 6 hours. For storage longer than 6 hours, store samples at -20 °C or below. Avoid repeated freeze/thaw cycles.
7. Use dry ice for transport.
8. Thaw samples on ice prior to analysis in the Merckodia Glucagon ELISAs, 10-1271-01 or 10-1281-01.

EDTA plasma samples

1. Collect blood by venipuncture into tubes containing EDTA as anticoagulant.
2. Upon collection, mix the contents gently (without shaking) by inverting the tube.
3. Collect the plasma fraction by centrifugation.
4. Use a pipette to carefully transfer the plasma fraction into appropriate vials.
5. Avoid storing samples at 2-8 °C for longer than 6 hours. For storage longer than 6 hours, store samples at -20 °C or below. Samples are stable at -80 °C for up to 12 months. Avoid repeated freeze/thaw cycles.
6. Use dry ice for transport.
7. Thaw samples on ice prior to analysis in the Merckodia Glucagon ELISAs, 10-1271-01 or 10-1281-01.

BD™ P800 plasma samples

1. Collect blood by venipuncture into Becton Dickinson (BD™) P800 tubes (Research Use Only) containing EDTA as anticoagulant, and protease, esterase and DPP-IV inhibitors.
2. Upon collection, mix the contents gently (without shaking) by inverting the tube.
3. Collect the plasma fraction by centrifugation.
4. Use a pipette to carefully transfer the plasma fraction into appropriate vials.
5. Avoid storing samples at room temperature or 2-8 °C for longer than 24 hours. For storage more than 24 hrs, store at -20 °C or below. Samples are stable at -20 °C or below for up to 12 months. Avoid repeated freeze/thaw cycles.
6. Use dry ice for transport.
7. Thaw samples on ice prior to analysis in the Mercodia Glucagon ELISAs, 10-1271-01 or 10-1281-01.

References

Wewer Albrechtsen *et al.* (2015) Stability of glucagon-like peptide 1 and glucagon in human plasma. *Endocrine Connections* 4: 50-57.

Zhu *et al.* (2003) The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides. *J Biol Chem* 278: 22418-22423.