Mercodia

Technical Note, No: 34-0154, v.2.0

Sample collection and handling for Mercodia Total GlP-1 NL-ELISA (10-1278-01)

Mercodia Total GLP-1 NL-ELISA measures active GLP-1 (7-36) amide to the same extent as its metabolite GLP-1 (9-36) amide. Thus, the rapid degradation of the active GLP-1 (7-36) amide form is not a problem for the specificity of the assay. Accurate results are obtained even if samples without any stabilizing additives are analyzed, therefore, both serum and EDTA plasma samples can be used in the assay.

Summary

- Avoid storing samples at room temperature or 2-8°C for long periods of time. (Figures 1 and 2).
- Avoid freeze/thaw cycles. (Figure 3).
- We recommend storing samples below -20°C (Figure 4).
- For studies in which very low levels of GLP-1 need to be detected, or if samples need to be exposed to room temperature, it may be beneficial to use BD™ (Becton Dickinson) P800 tubes (Article no. 366420) for sample collection since this will prevent GLP-1 degradation.
- For details on sample collection, see pages 11-12.

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Short-term stability

At 2-8°C, GLP-1 in serum or EDTA plasma is stable for at least 24 hours (Figure 1).

After 24 hours at room temperature (RT), serum GLP-1 concentrations were significantly reduced. However, both normal EDTA plasma and P800 plasma samples were stable for as long as 24 hours at RT (Figure 2).

These findings are consistent with Wewer Albrechtsen *et al.* (2015) who reported that the metabolite GLP-1 (9-36) amide seemed stable at RT for at least 3 hours. In the same study, it was clear that intact GLP-1 (7-36) amide did not show the same stability, with concentrations significantly reduced after 3 hours at RT. The authors discussed the importance of using tubes containing DPP-IV inhibitors when measuring intact (or active) GLP-1.

Our data support the advantage of using a total GLP-1 assay whenever possible to reduce pre-analytical errors and expand sample type compatibility.

Mercodia GLP-1 NL-ELISA measures both GLP-1 (7-36) and GLP-1 (9-36) amides to the same degree (Table 1). Even if DPP-IV degrades the intact GLP-1 (7-36) to the metabolite GLP-1 (9-36) form, the measured concentration will still be the same in the Mercodia assay.

Table 1. Specificity of Mercodia Total GLP-1 NL-ELISA (10-1278-01).

	Cross-reaction
GLP-1 (1-36) amide	88%
GLP-1 (7-36) amide	93%
GLP-1 (9-36) amide	100%

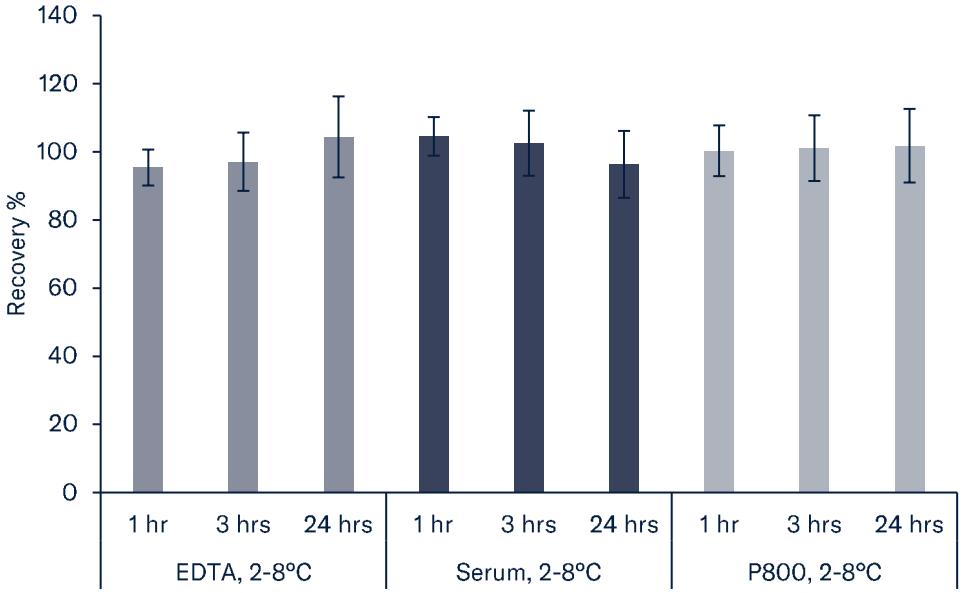

Figure 1. Sample stability at 2-8°C for 1, 3 and 24 hours for both serum and EDTA plasma as well as samples collected in P800 tubes. Mean recoveries from ten individual samples (± standard error of the mean) are presented.

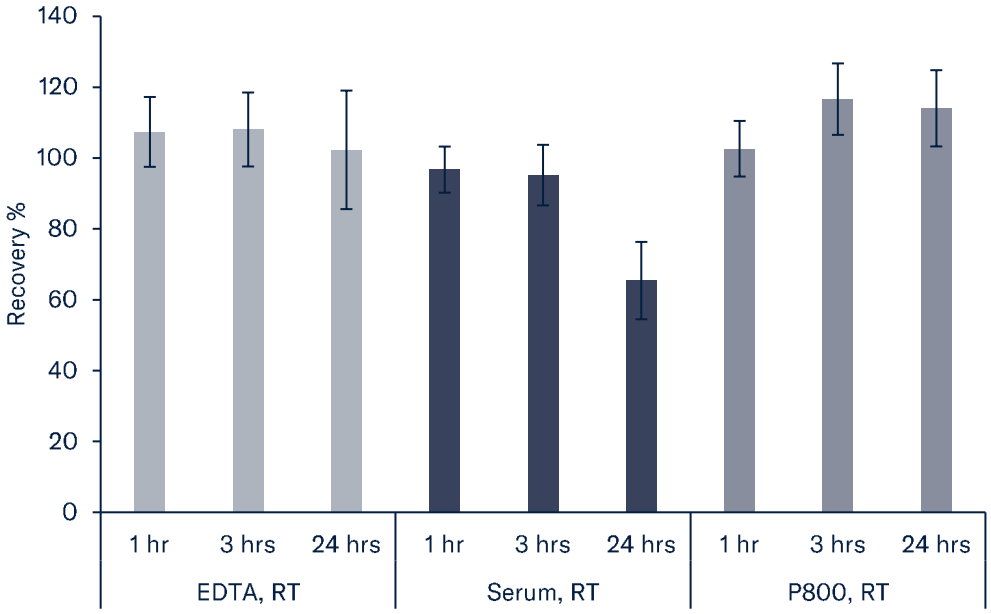

Figure 2. Sample stability at room temperature (RT) for 1, 3 and 24 hours for both serum and EDTA plasma as well as samples collected in P800 tubes. Mean recoveries from ten individual samples (± standard error of the mean) are presented.

Freeze/thaw stability

Wewer Albrechtsen et al. (2015) showed that the concentrations of both GLP-1 (7-36) and GLP-1 (9-36) amides were not significantly changed by up to three repeated freeze/thaw cycles. These results were confirmed in the Mercodia in-house study (Figure 3).

Certainly, sample-specific difference cannot be excluded and, therefore, multiple freeze/thaw cycles should be avoided whenever possible. We recommend aliquoting samples in appropriate tubes and volumes before use.

Long-term stability

A long-term stability study for GLP-1 in serum and EDTA plasma (including samples collected in P800 tubes) is currently ongoing at Mercodia. Data from Wewer Albrechtsen *et al.* (2015) suggests that GLP-1 is stable for at least 12 months both at -20°C and -80°C, Figure 4.

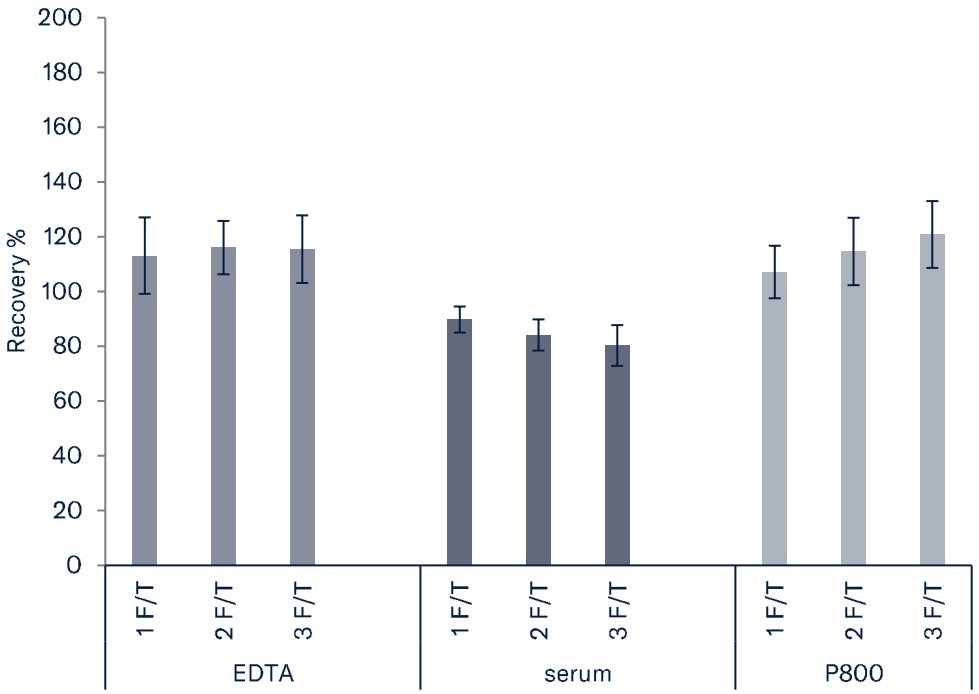

Figure 3. Sample stability after freeze/thaw cycles for both serum and plasma samples. Mean recoveries from ten individual samples (± standard error of the mean) are presented.

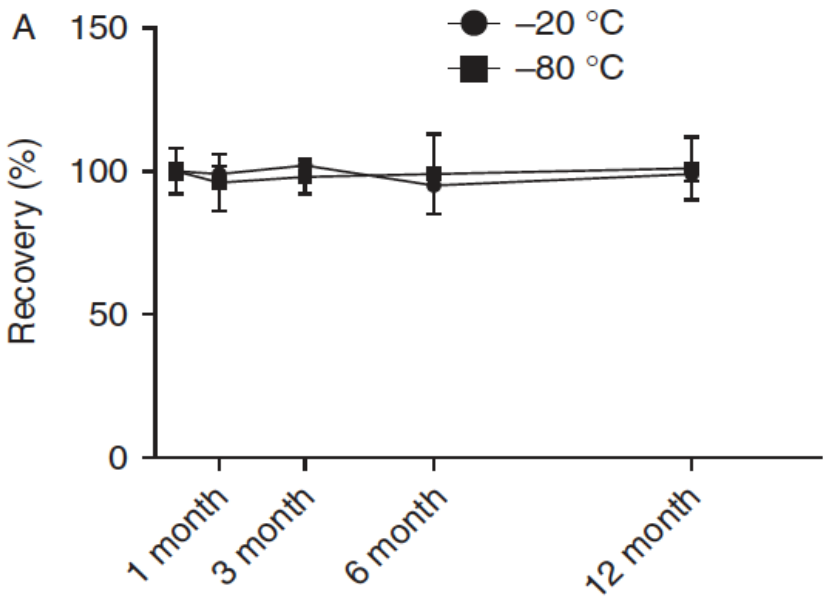

Figure 4. Impact of long-term storage at -20°C (circles) and -80°C (squares) on the recovery of added (40 pmol/L) GLP-1 (7-36) amide spiked in human plasma. Each result represents the mean ± sd of eight replicated determinations.

References

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

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 of sample processing, please refer to the instructions provided by the tube manufacturer.

Serum samples

1. Collect blood by venipuncture into serum tubes.
2. 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 by centrifugation immediately after clotting.
5. Use a pipette to carefully transfer the serum into appropriate vials.
6. Avoid storing samples at 2-8°C for longer than 24 hours. For longer durations, 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 with Mercodia Total GLP-1 NL-ELISA (10-1278-01).

EDTA plasma samples

1. Collect blood by venipuncture into tubes containing EDTA as anticoagulant.
2. 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 24 hours. For longer durations, store samples at -20°C or below. Avoid repeated freeze/thaw cycles.
6. Use dry ice for transport.
7. Thaw samples on ice prior to analysis with Mercodia Total GLP-1 NL-ELISA (10-1278-01).

BD™ P800 plasma samples

1. Collect blood by venipuncture into Becton Dickinson (BD™) P800 tubes (Research Use Only) containing EDTA as anticoagulant, protease, esterase and DPP-IV inhibitors.
2. 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 longer durations, store at -20°C or below. Avoid repeated freeze/thaw cycles.
6. Use dry ice for transport.
7. Thaw samples on ice prior to analysis with Mercodia Total GLP-1 NL-ELISA (10-1278-01).