SAMPLE COLLECTION AND HANDLING FOR THE MERCODIA GLUCAGON ELISA (10-1271-01)

SUMMARY

In the Mercodia Glucagon ELISA, both serum and EDTA plasma can be used. However, glucagon in serum or EDTA plasma samples will be sensitive to storage conditions and freeze/thaw cycles.

For details about sample collection, please see pages 11-12.

- Avoid storing samples at room temperature or 2-8°C for long periods of time since glucagon is degraded (Figure 1).

- We recommend that samples be stored at -80°C (Figures 2 and 7).

- Avoid freeze/thaw cycles. (Figures 3 and 5).

- The addition of aprotinin to samples will not improve the stability of glucagon (Figure 4).

- For studies in which very low levels of glucagon need to be detected, it may be beneficial to stabilize plasma, e.g. by using tubes such as BD™ (Becton Dickinson) P800 for sample collection (art nr 366420), since this will prevent the degradation of glucagon (Figure 6).

- If samples are to be stored, we recommend storing them as serum or plasma rather than as whole blood (Figure 9).

- Sample extraction does not improve glucagon yield (Figure 10).
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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.

Figure 1. Sample stability over time at different storage conditions for both serum and EDTA plasma samples.
**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 ± 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).

![Graph showing long-term stability with average recoveries from 12 EDTA samples stored at -20°C or -80°C.](image)

*Figure 2. Long-term stability with average recoveries from 12 EDTA samples stored at -20°C or -80°C.*
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 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, Mercodia strongly suggests that samples 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.

Figure 4. Sample stability over time at different storage conditions for EDTA plasma with addition of aprotinin.
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 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.

Figure 6. Sample stability over time at different storage conditions for EDTA plasma in P800 tubes.
**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.

*Figure 7. Long-term stability with average recoveries from 12 P800 plasma samples stored at -20°C or -80°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 provided that samples are stored at -80°C and kept on ice when thawed (Figure 8). Since sample specific differences cannot be excluded, Mercodia recommends avoiding freeze/thaw cycles if possible.

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. Sample stability. 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.
SAMPLE EXTRACTION

Sample extraction does not improve glucagon yield in EDTA plasma (Figure 10).

Figure 10. 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 Mercodia Glucagon ELISA, 10-1271-01.

**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 Mercodia Glucagon ELISA, 10-1271-01.
**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 ELISA, 10-1271-01.

**REFERENCES**
