Mercodia

Technical Note, No: 34-161, v.1.0

For Research Use Only. Not for use in diagnostic procedures.

Simultaneous protocol for Mercodia Glucagon ELISA (10-1271-01)

The Mercodia Glucagon ELISA (10-1271-01) was launched in 2013 as a simultaneous protocol with high specificity to glucagon and below 1 % cross reactivity to the proglucagon derivative glicentin. However, it was found that under certain conditions such as post bariatric surgery the concentrations of glicentin can be elevated to levels that may interfere in the assay[1]. To abolish this interference, a sequential protocol was developed and is now the standard procedure for the assay (from October 1st 2020). The original protocol can still be used with the help of this Technical Note.

The Mercodia Glucagon ELISA is validated with a sequential assay protocol. See specifications in the Directions for Use for the Mercodia Glucagon ELISA 10-1271-01. However, for customers wishing to run the original, simultaneous protocol instructions within this Technical Note can be followed.

Sample correlation between the two protocols is good (Figure 1) and the average recovery for Dilutional Linearity and Parallelism is within the accepted range for both protocols.

PLEASE OBSERVE:
For regulatory purposes, this protocol is for research use only (RUO). Using this protocol will leave one bottle of Assay Buffer in the kit unused.

References

1. Roberts, G. P. *et al.* Gastrectomy with Roux-en-Y reconstruction as a lean model of bariatric surgery. *Surg. Obes. Relat. Dis.* (2018). doi:10.1016/j.soard.2018.01.039

Summary Protocol Sheet

Please read current Directions for Use for the Mercodia Glucagon ELISA (10-1271-01) for more information before performing the assay.

Add Calibrators, Controls, and samples	25 µL
Add enzyme conjugate 1X solution and attach plate sealer	200 µL
Incubate	O/N (18-22h) at 2-8°C on a plate shaker (700-900 rpm)
Wash plate with wash buffer 1X solution	700 µL, 6 times*
Add Substrate TMB	200 µL
Incubate	15 min at 18-25°C on the bench
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A_{450}	Evaluate results. Preferably use 5-parametric logistic regression with automatic weighing on relative weights ($1/Y^2$).

* Wash 6 times with 700 µL wash buffer 1X solution per well, using an automatic plate washer with an overflow wash function. After the final wash, invert and tap the plate firmly against absorbent paper. Do not include a soak step in the washing procedure. For manual washing, see Technical Note No: 34-0106 Instruction for manual washing procedure for microplates (available online).

Performance Characteristics

Sensitivity and Range of Quantification

The detection limit is 1 pmol/L as determined by the methodology described in ISO11843-Part 4.

Lower Limit of Quantification, LLOQ, is 1.7 pmol/L as determined according to FDA/EMA guidelines.

The Upper Limit of Quantification, ULOQ, is 130 pmol/L as determined according to FDA/EMA guidelines.

Parallelism

P800 samples spiked with Glucagon to high concentrations within the measuring range were diluted 1/2, 1/4 and 1/8. Mean recovery for parallelism is 91 % (78-112 %) with precision between samples in the dilution series ≤ 15 %.

Dilutional Linearity

P800 samples were spiked above the highest calibrator concentration and subsequently diluted for analysis in the assay. Nominal values were used for calculation. Mean recovery for dilutional linearity is 83 % (80-87%) with a precision of the final concentration across all dilutions of 6 %.

Cross-reactivity

The simultaneous protocol has a slightly higher cross reactivity to certain proglucagon derivates compared to the sequential protocol, see Table 1. This cross reactivity is not an issue in most patient groups, where levels of proglucagon derivatives are low, but when investigating samples where levels of glicentin can be expected to be very high, we strongly recommend using the protocol described in the current version of Directions for Use for the Mercodia Glucagon ELISA 10-1271-01.

Table 1. Specificity of the Mercodia Glucagon ELISA using the simultaneous assay protocol.

	Cross-reactivity (%)	Conc. tested (pmol/L)
Glicentin	0.8	300
Oxyntomodulin	4.4	135

Sample correlation

Correlation between the two different assay protocols is high with an R^2-value above 0.96, see Figure 1. The mean difference between the two protocols is ≤20 %.

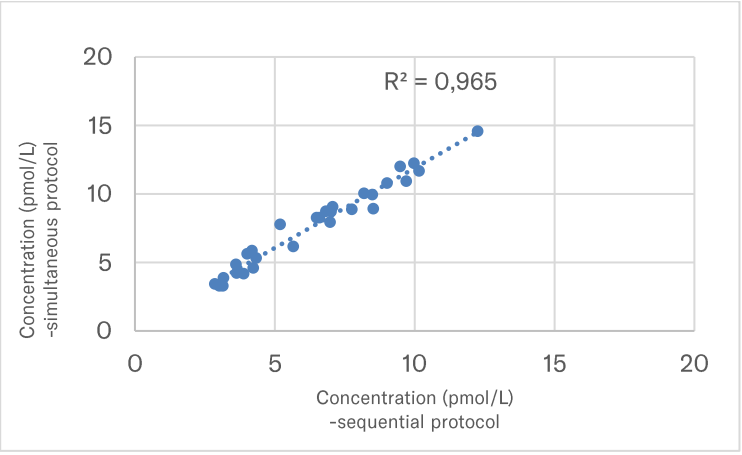

Figure 1. Correlation plot including 29 P800 plasma samples in duplicate