Mercodia

Technical Note, No: 34-162, v.2.0

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

Alternative sequential protocol for increased specificity in Mercodia Glucagon ELISA - 10 µL (10-1281-01)

Under certain conditions like post bariatric surgery, the concentrations of glicentin, have been found to be elevated to levels that might interfere in the human Glucagon ELISA (10-1271-01) [1]. To abolish this interference, a sequential protocol was developed and is now the standard procedure for the human assay (from October 1st 2020). For situations where also glicentin might be exceptionally high when using the Glucagon ELISA - 10 µL (10-1281-01), this Technical Note can be used to achieve the highest possible specificity.

The Mercodia Glucagon ELISA -10µL (10-1281-01) is validated with a simultaneous assay protocol. (See specifications in the Directions for Use for the Mercodia Glucagon ELISA – 10 µL, 10-1281-01.)

A modification of the protocol is here suggested that will improve specificity when there are exceptionally high concentrations of glicentin in the sample. By introducing a sequential protocol un-specifically bound material will be washed away from the antibody-coated plate, without losing sensitivity.

PLEASE OBSERVE
This protocol requires one extra vial of Enzyme Conjugate Buffer. Therefore, two kits are required to run 42 samples in duplicates. The kits can be ordered by emailing info-global@mercodia.com or by contacting your local sales representative.

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 the original Directions for Use for the Mercodia Glucagon ELISA - 10 µL (10-1281-01) for more information before performing the assay.

Add Calibrators, Controls and samples	**10 µL**
Add Enzyme Conjugate Buffer (art no 20-7153) to all wells	50 µL
Incubate	O/N (18-22h) at 4°C on a plate shaker (700-900 rpm)
Wash plate with wash buffer 1X solution	6 times*
Add enzyme conjugate 1X solution to all wells	50 µL
Incubate	1 h at 18-25°C on a plate shaker (700-900 rpm)
Wash plate with wash buffer 1X solution	6 times*
Add Substrate TMB	200 µL
Incubate	30 minutes at 18-25°C on the bench
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A450	Evaluate results

* 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 at www.mercodia.com).

Performance Characteristics

Cross reactivity

The sequential assay protocol minimizes cross-reactivity from glicentin (Table 1)

Table 1. Cross-reactivity (%) of rat glicentin at different concentrations in the Mercodia Glucagon ELISA - 10 µL,10-1281-01, using the simultaneous protocol in the DfU vs. the Sequential protocol as described in this Technical Note

Rat Glicentin (pM)	Cross-reactivity (%)	
	DfU	Sequential Protocol
2840	n.d	0.4
2130	6.5	0.3
1598	4.9	n.d
1198	4.4	n.d
899	3.8	n.d
674	3.5	n.d
505	2.8	n.d

n.d = not detected, below or over assay range

Protocol correlation

Correlation between the normal (DfU) and the Sequential protocol is good, as can be seen from measurements of dilutions of the highest calibrator (Calibrator 5), included in the Glucagon ELISA Mercodia - 10 µL, 10-1281-01 (Figure 1).

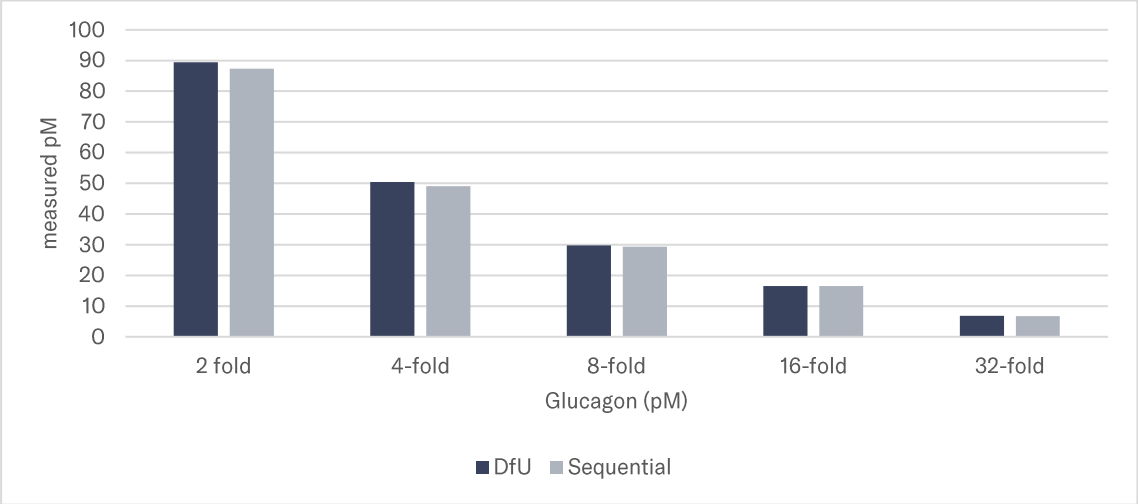

Figure 1. Dilution series of Calibrator 5 in Mercodia Glucagon ELISA - 10µL 10-1281-01. Calibrator 0 was used as diluent.