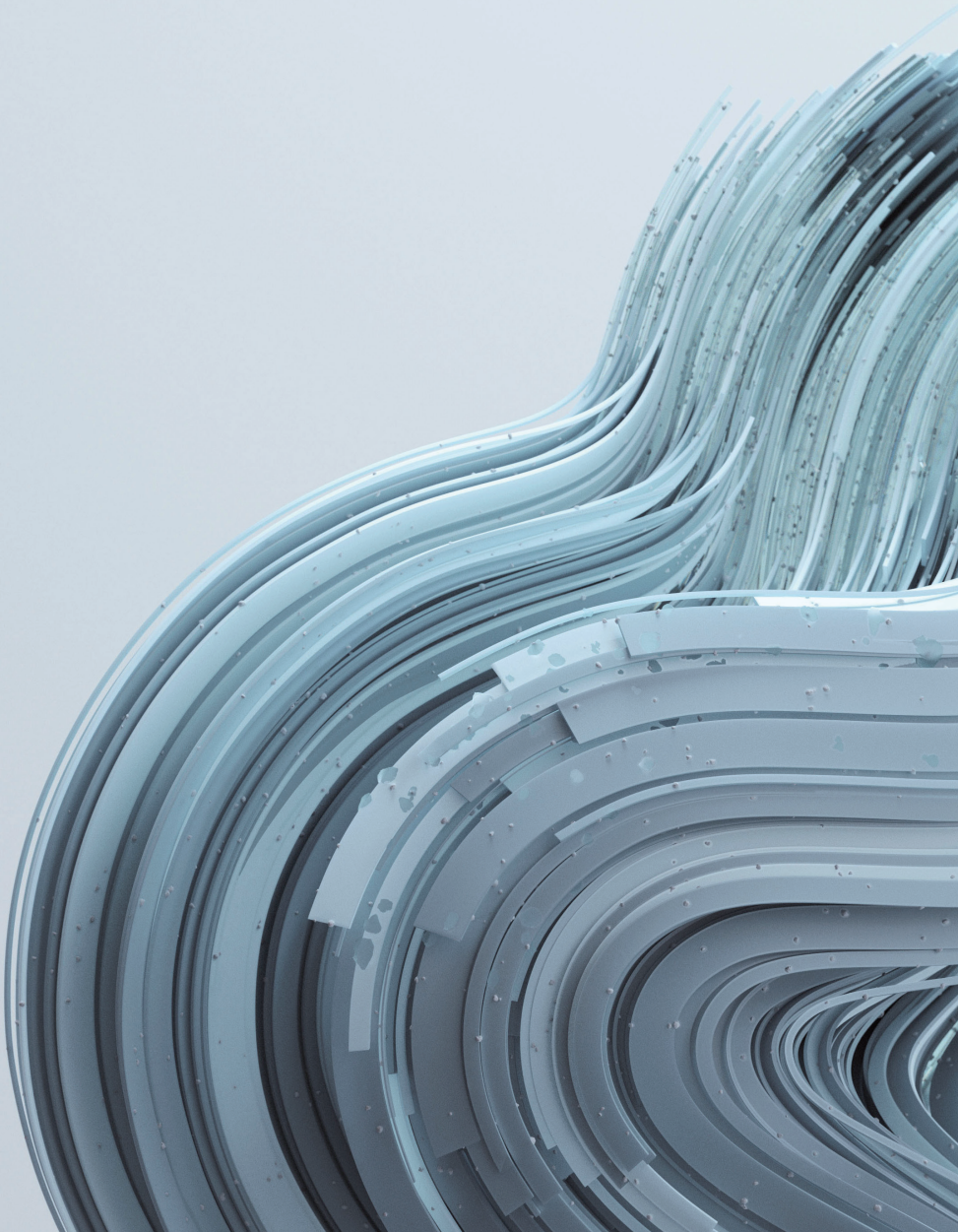
Mercodia

How to choose your Glucagon Assay

The Mercodia Glucagon ELISA has been used in more than 75 peer-reviewed scientific articles from researchers around the world and is now considered by many to be the gold standard for glucagon measurements. Inaccurate glucagon readings can have enormous negative implications on research and clinical decisions, which is why it is important to carefully choose your glucagon assay before starting any experiments.

The Mercodia Glucagon ELISA is known for its **superior sensitivity**, **high specificity** and **easy-to-use protocol** without sample pre-treatment or radioactivity.

In this brochure, we will tell you more about what distinguishes the Mercodia Glucagon ELISAs from all other glucagon ELISAs, and give you some tips on what to look for in a good glucagon assay.



When accuracy matters
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Superior Sensitivity - Proven by Independent Evaluators

Recently, new commercial assays for glucagon have appeared on the market. To avoid wasting money on an assay that doesn't fit your needs, make sure to ask about the analytical sensitivity of the assay before purchasing. Many assays have a measurement range set much broader than the assay can measure with any kind of accuracy, and the lower limit at which the assay can provide quantitative results (LLOQ) is, in reality, much higher than what is stated.

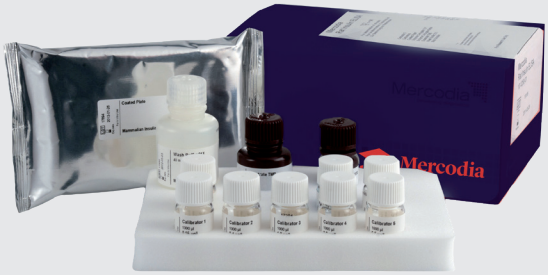
The Mercodia Glucagon ELISA has superior sensitivity, which enables you to measure physiologically suppressed levels of glucagon. This has been proven by independent evaluators, stating that only the Mercodia Glucagon ELISA, when compared to Millipore and Meso Scale Discovery assays, had optimal performance for measuring glucagon concentrations in clinical samples (Wewer Albrechtsen et al., 2016).

In order to provide the scientific community with the ability to detect glucagon under various physiological conditions, Mercodia offers two novel glucagon assays with superior measuring ranges, compared with other assays on the market. The Mercodia Glucagon ELISAs for human and animal samples have sensitivities of 1.5 pmol/L (5 pg/mL) and 2 pmol/L (7 pg/mL), respectively. These assays meet the needs of researchers as discussed by Bak et al., who state that test methods with sensitivities >5 pmol/L (17 pg/mL) do not allow for full characterization of glucagon (Bak MJ et al., 2014).

The Mercodia Glucagon ELISA is validated according to FDA/EMA guidelines and has a full bioanalytical validation report that can be acquired from Mercodia.

"Of further concern is the finding that some of the kits that we purchased appeared to be incapable of quantifying what they purported to."

(Wewer Albrechtsen et al., 2016)



Am I getting accurate glucagon values? High Specificity - With Full Transparency for Our Customers

When choosing a glucagon immunoassay, make sure to take a few minutes and study the cross-reactivity table carefully. If you are interested in measuring active glucagon (1-29), the assay should not cross-react with the metabolite, glucagon 3-29 (Campbell & Drucker, 2015). Any cross-reactivity to the proglucagon-derived gut peptides oxyntomodulin and glicentin should also be low and if present, well described, because they contain the full glucagon sequence. Glicentin circulates at much higher levels than glucagon, and therefore cross-reactivity to glicentin can create serious measurement error when trying to measure glucagon.

The Mercodia Glucagon ELISA has very low cross-reactivity to oxyntomodulin and glicentin, and data on the cross-reactivity at different concentrations can be obtained from Mercodia. Biacore and ELISA analyses have shown that there is no cross-reactivity to glucagon 3-29, at or above physiological concentrations, in the Mercodia Glucagon ELISA. This is an important finding since glucagon 3-29 has been reported to be the major metabolite in clinical samples due, in large part, to in vitro plasma protease metabolism during sample storage (Howard JW et al., 2015). Increased specificity is a primary reason why concentrations in the Mercodia glucagon assays are lower than concentrations generated by other glucagon assays.

Issues with specificity raise questions about true glucagon concentrations reported by assays that cross-react with glicentin or for which the manufacturer chooses not to report cross-reactivity to relevant homologous proteins. The use of an assay that cross-reacts with other glucagon-containing proteins may result in inaccurate

characterization of alpha cell function and glucagon kinetics under various physiological conditions, as well as misinterpretation of how a subject is responding to therapy. Falsely elevated levels will also have a negative impact on decisions about inclusion of subjects in studies or on stratifications based on specific glucagon cut-off values.
If you work with samples from gastric bypass surgery patients or if you infuse high levels of gut hormones, contact Mercodia so that we can provide you with an alternative protocol for the Glucagon ELISA, which will result in even further increased specificity!

Don't forget to also pay attention to how cross-reactivity studies have been performed. Always check to see what concentrations were used in a manufacturer's cross-reactivity testing, since the level of cross-reactivity is usually positively correlated to the concentrations tested. If levels are not stated, be suspicious!

My sample volumes are limited – is there an assay that can meet my needs?

Sample volume requirements have significantly limited the use of glucagon assays. Most commercially available methods require at least 50-100 µL of plasma. This has direct implications on the number of analytes that can be measured, the number of time points that can be examined and thus, the scope of scientists' conclusions.

The Mercodia Glucagon ELISAs were developed and optimized to require minimal sample volumes, with the assay for human samples requiring only 25 µL, and the assay for animal samples requiring only 10 µL of sample. These very low sample volumes offer a significant advantage over the requirements of traditional assays and enable scientists to measure glucagon along with other relevant hormones, and/or study temporal changes in glucagon in a variety of experimental models.

Will matrix interferences affect my results?

The amino acid sequence of glucagon is highly conserved across species, but validation of different sample types is crucial because sequence homology is not the only factor that affects the antibody-antigen binding in an immunoassay. For example, matrix interferences (common in animal samples) can lead to falsely elevated or falsely low concentrations. According to Bak et al., some assays in their evaluation of human glucagon ELISAs from 2014 had poor recovery in plasma and/or buffer. The assays in this evaluation reported variable baseline concentrations and glucagon was not always detected, particularly under conditions in which it was suppressed.

Mercodia assays contain a unique blocking solution to prevent or minimize matrix interferences, especially important for the 10 µL assay, which has been optimized for animal samples.
Both of the Mercodia Glucagon assays can be used with samples from cultured cells, providing accurate glucagon measurement options for those conducting in vitro research (e.g., conversion of alpha to beta cells or vice versa, human embryonic stem cells to alpha or beta cells, production of islet-like clusters, etc.).

Can I trust my results using different batches of the assay? Measurement Quality - Leading the Field

Many assays produced with insufficient production methods will have edge effects and lack of homogeneity across the plate, giving rise to high variation between samples depending on plate position. Always check plate homogeneity with a sample in the lower segment of your measuring range (this is where this problem becomes the most apparent) to make sure the assay fits your purpose.

There are many factors that could potentially influence the results from an immunoassay. It is extremely important to control every step of the manufacturing process, from antibody to final product, and this is why Mercodia only uses monoclonal antibodies. For the Mercodia Glucagon assays, two highly specific mouse monoclonal antibodies were developed and characterized extensively using ELISA and Biacore techniques. The 25uL Mercodia Glucagon ELISA is validated for human samples and the 10 µL Mercodia Glucagon ELISA has been validated for mouse, rat, and non-human primate samples.

The antibodies are controlled and manufactured with the same characteristics in each lot, resulting in assays with very low lot-to-lot variation, year after year.

The Mercodia Quality System

The Mercodia Glucagon ELISA is CE/IVD marked and produced under strict control of the Mercodia Quality System (MQS).

- ISO certified
- QSR governed SOPs
- Documentation & traceability
- State-of-the-art instrumentation
- Routine instrument calibration and maintenance
- Life Cycle Management

References

Bak MJ et al. (2014) Specificity and Sensitivity of Commercially Available Assays for Glucagon and Oxyntomodulin Measurement in Humans. Eur J Endocrinol 170:529-538.

Campbell, J. E., & Drucker, D. J. (2015). Islet a cells and glucagon—critical regulators of energy homeostasis. Nature Reviews Endocrinology, 11(6), 329–338.

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