

Quantification of functional GLP-1 using *iLite*[®] GLP-1 Assay Ready Cells

For research and professional use only. Not for use in diagnostic procedures.

*This application note contains a suggested protocol and performance data.
Each individual laboratory must set up their own method and perform relevant validations.*

Background

Glucagon-like peptide-1 (GLP-1) is an incretin hormone belonging to the secretin/vasoactive intestinal polypeptide family of gastrointestinal regulatory peptides. It is a 30-aminoacid peptide produced primarily by enteroendocrine L-cells of the distal small intestine and colon in response to nutrient ingestion (1,2). GLP-1 is derived from the tissue-specific post-translational processing of the proglucagon gene and exerts its biological effects through the GLP-1 receptor (GLP-1R) (1,2).

Physiologically, GLP-1 plays a central role in the regulation of glucose homeostasis and energy balance by enhancing glucose-dependent insulin secretion, suppressing glucagon secretion, slowing gastric emptying, and promoting satiety through actions in the central nervous system (1,2). These coordinated effects reduce postprandial glucose excursions while helping regulate food intake and body weight (3). Incretin-based therapeutics leveraging the function of GLP-1 have evolved to become highly effective and widely applied medications to treat type 2 diabetes and obesity complicated by comorbidities (4). Current incretin-based therapies comprise GLP-1 receptor agonists (e.g., semaglutide) and newer dual GIP-GLP-1 receptor co-agonists (e.g., tirzepatide), which leverage complementary hormonal pathways to treat hyperglycemia in people with type 2 diabetes and promote body weight reduction (5,6). Dual agonists represent an evolution of GLP-1 therapies, combining GIP and GLP-1 receptor activation to achieve greater metabolic efficacy and form the basis of emerging next-generation anti-obesity pharmacotherapies (5,7).

The *iLite*[®] GLP-1 Assay Ready Cells can be used for functional studies of GLP-1, the receptor GLP-1R and their interaction.

Principle of the assay

The *iLite*[®] GLP-1 Assay Ready Cells are engineered cells, optimized to be responsive to GLP-1 receptor agonists, resulting in a proportional expression of Firefly Luciferase (FL). The binding of GLP-1 to its receptor results in activation of the CRE regulated Firefly luciferase reporter gene construct. *iLite*[®] GLP-1 Assay Ready Cells also contain the Renilla Luciferase (RL) reporter gene, under the control of a constitutive promoter. The constitutive expression of RL allows normalization of GLP-1 induced FL activity, and renders assay results independent of variations in cell number or serum matrix effects. The luciferase signal can be measured in a luminometer following addition and incubation of luciferase substrate. The Firefly luciferase signal is proportional to the concentration of functional GLP-1 in a sample (Fig.1).

Specimen collection

The *iLite*[®] GLP-1 Assay Ready Cells can be used for measuring concentration of GLP-1 receptor agonists in test samples including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
<i>iLite</i> [®] GLP-1 Assay Ready Cells	Svar Life Science	BM4100
Diluent (DMEM containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Liraglutide or other GLP-1 receptor agonists	Novo Nordisk	NA
Firefly/Renilla luciferase substrate	Promega	E2920, Dual-Glo Luciferase Assay System
Plate; White walled micro well plate suitable for luminescence	Revvity	6055680
Microplate Luminometer with appropriate reading software – no filter on luminometer	Contact Svar Life Science for list of recommended suppliers	NA
Incubator, 37 °C with 5% CO ₂	NA	NA
Water bath, 37 °C	NA	NA
Single-channel and multi-channel pipettes with polypropylene disposable tips	NA	NA
Polypropylene tubes or plate for dilution	NA	NA
Single-use polypropylene reservoir	NA	NA
Plate shaker	NA	NA
Timer	NA	NA

Protocol

Preparation of calibrators (GLP-1 receptor agonist)

The peptide Liraglutide from Novo Nordisk has been used to stimulate the *iLite*[®] GLP-1 Assay Ready Cells. The table below shows the dilutions of Liraglutide, used for QC release of the *iLite*[®] GLP-1 Assay Ready Cells.

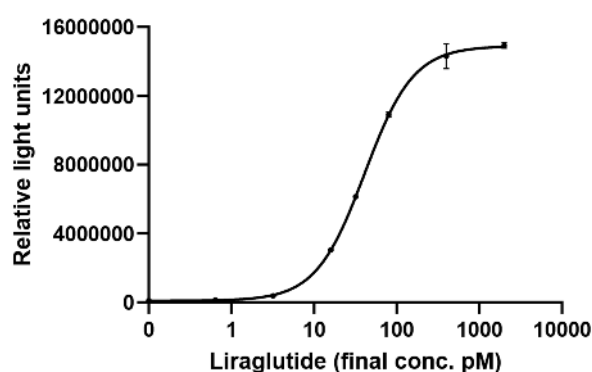


Figure 1. Example of Liraglutide calibration curve.

Calibrator	Liraglutide Suggested calibrator solution conc. (pM)
A	4000
B	800
C	160
D	64
E	32
F	6.4
G	1.3
H	0

Table 1. Suggested calibrator solution concentrations for Liraglutide.

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicates.
2. Dilute calibrators, controls, and samples to fall within the expected in assay values of 0 - 2000 pM.
3. Add 40 µL calibrators, controls, and samples in duplicate to assigned wells (final concentration will be half of solution concentration).

4. Thaw the vial of *iLite*[®] GLP-1 Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully ten times with pipette to ensure a homogeneous distribution of cells.
5. Dilute 250 µL cell suspension with 5.75 mL Diluent.
6. Add 40 µL diluted cells to each well.
7. Place the lid on the plate, mix and incubate for 5 hours at 37 °C with 5% CO₂.

Adding substrate solutions

8. Equilibrate the plate and the substrate solution to room temperature.
9. Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.
10. If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.

Precautions

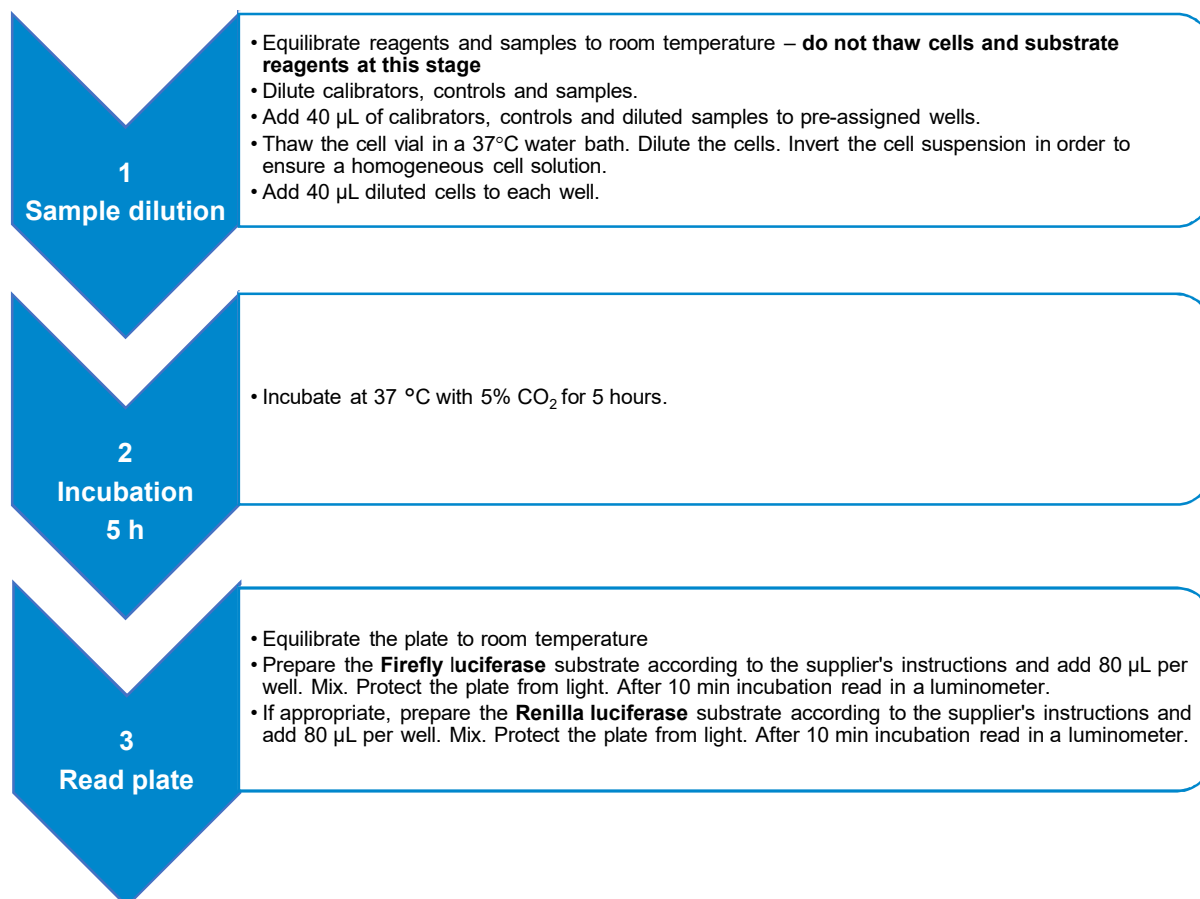
- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according to the supplier's/manufacturer's instructions or product specifications accompanying the individual material and instruments.
- Dispose of all sample specimens, infected or potentially infected material in accordance with good microbiological practice. All such materials should be handled and disposed as potentially infectious.
- Residues of chemicals and preparations are generally considered as biohazardous waste and should be inactivated prior to disposal by autoclaving or using bleach. All such materials should be disposed in accordance with established safety procedures.

Proprietary Information

In accepting delivery of *iLite*[®] Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third-party recipient, and only to use them directly in assays. *iLite*[®] cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered *iLite*[®] Assay Ready Cells is an infringement of these patents.

QUICK GUIDE

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Troubleshooting and FAQ

Please consult the Svar Life Science website www.svarlifescience.com

References

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