Quantification of GLP-1 inhibitory activity 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 measurements of GLP-1 inhibitor activity / GLP-1 receptor inhibitor activity.

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 Firefly luciferase signal can be measured in a luminometer following addition and incubation of luciferase substrate. The Firefly luciferase signal is proportional to the functional activity of GLP-1 receptor agonist in the sample. In the presence of GLP-1 receptor inhibitor or a substance able to neutralize activity of GLP-1 receptor agonist, the signaling through GLP-1 receptor is reduced, resulting in a decreased stimulation of Firefly luciferase production.

Thus, the Firefly luciferase signal is inversely proportional to the amount of inhibitory activity against either GLP-1 receptor or GLP-1 receptor agonist in a sample. The *iLite*® GLP-1 Assay Ready Cells can

therefore be utilized as an assay for quantification of GLP-1 inhibitor activity / GLP-1 receptor inhibitor activity in test samples, including human serum. In the following outline, anti-GLP-1 receptor antibody is used as example of GLP-1 receptor inhibitory activity.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® GIP Assay Ready Cells	Svar Life Science	BM4110
Diluent (DMEM containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin).	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Inhibitory anti-GLP-1 receptor antibody	DSHB	MAb 3F52
Liraglutide	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

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 though 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 of in accordance with established safety procedures.

Propriety 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

Protocol

Preparation of GLP-1 receptor inhibitor

Anti-GLP-1 receptor monoclonal antibody from the Developmental Studies Hybridoma Bank (DSHB) have been used to neutralize stimulation induced by Liraglutide and inhibit signaling through GLP-1 receptor in *iLite*® GLP-1 Assay Ready Cells (refer to the table and graph below).

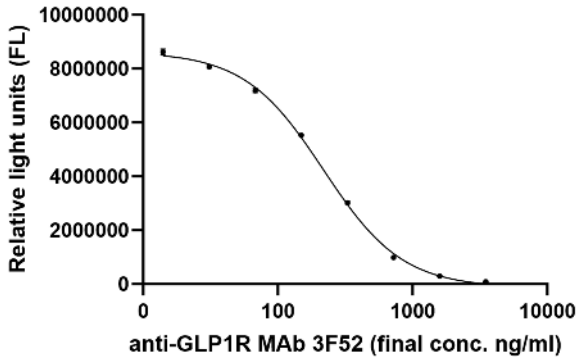

Figure 1. Example of anti-GLP-1 receptor MAb dose-response-curve

Final 50 pM Liraglutide	Anti-GLP-1R MAb Suggested calibrator solution concentrations, ng/mL
A	14000
B	6364
C	2893
D	1315
E	598
F	272
G	123
H	56

Table 1. Suggested calibrator solution concentrations for anti-GLP-1R MAb cat# 3F52

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at minimum in duplicates.
2. Perform a serial dilution of the reference GLP-1R inhibitor. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference GLP-1R inhibitor dilutions, controls and samples to assigned wells (final concentration will be a quarter of the 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 2.75 mL Diluent.
6. Add 20 µL diluted cells to each well.
7. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37 °C with 5% CO_2.
8. Add 40 µL of GLP-1 receptor stimulator (here Liraglutide) to all wells (with solution concentration at 100 pM, final concentration will be 50 pM Liratide).
9. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37 °C with 5% CO_2
10. 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.
11. Dilute 250 µL cell suspension with 5.75 mL Diluent.
12. Add 40 µL diluted cells to each well.
13. Place the lid on the plate, mix and incubate for 5 hours at 37 °C with 5% CO_2.

Adding substrate solutions

14. Equilibrate the plate and the substrate solution to room temperature.
15. 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.

16. 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.

QUICK GUIDE

Quantification of GLP-1 receptor inhibitor activity using *iLite*® GLP-1 Assay Ready Cells

1 Sample dilution plus cell addiiton

- Equilibrate reagents and samples to room temperature – **do not thaw cells and substrate reagents at this stage**
- Dilute reference IL-GLP-1R inhibitor, controls and samples.
- Add 20 µL of ref. IL-GLP-1R inhibitor solutions, controls and diluted samples to pre-assigned wells.
- Thaw the cell vial in a 37°C water bath. Mix cell suspension with a pipette in order to ensure a uniform cell suspension. Dilute the cells.
- Add 20 µL diluted cells to each well.

2 Incubation 30 min

- Incubate at 37 °C with 5% CO_2 for 30 min.

3 Add Liraglutide

- Add 40 µL Liraglutide to each well.

4 Incubation 5 h

- Incubate at 37 °C with 5% CO_2 for 5 hours.

5 Read plate

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

Troubleshooting and FAQ

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

References

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