

Quantification of Insulin using *iLite*[®] Insulin 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

Insulin is a peptide hormone produced by beta cells in the pancreas to regulate the metabolism of carbohydrates and fats. Insulin is provided within the body in a constant proportion to remove excess glucose from the blood. When control of insulin levels fails, diabetes mellitus can result. As a consequence, insulin is used medically to treat some forms of diabetes mellitus (1).

Principle of the assay

The *iLite*[®] Insulin Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of an Insulin responsive promoter. Insulin exerts its activity by binding to a high affinity heterodimeric receptor, CD220, which possesses intrinsic tyrosine kinase activity. Binding of insulin to the insulin receptor alpha chain, results in receptor dimerization, receptor auto-phosphorylation, and signalling via the IR beta chain and activates the Insulin regulated Firefly luciferase reporter gene construct.

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 concentration of Insulin in the sample (Fig.1).

Specimen collection

The *iLite*[®] Insulin Assay Ready Cells can be used for measuring concentration of Insulin in test samples including human serum.

Material and equipment needed

| Material and equipment | Suggested supplier | Reference |
|---|---|--|
| <i>iLite</i> [®] Insulin Assay Ready Cells | Svar Life Science | BM3060 |
| Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin) | Gibco | 61870-044 (RPMI) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin) |
| Insulin or analogues | Life Technologies Inc. | 12585-014 |
| Firefly/Renilla luciferase substrate | Promega | E2920, Dual-Glo Luciferase Assay System |
| Plate; White walled micro well plate suitable for luminescence | PerkinElmer | 6005680 |
| 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
Timer

NA
NA

NA
NA

Protocol

Preparation of calibrators (Insulin)

Insulin from Life Technologies Inc. have successfully been used to stimulate the *iLite*[®] Insulin Assay Ready Cells. The below table shows the dilutions of Insulin, used for QC release of the *iLite*[®] Insulin Assay Ready Cells.

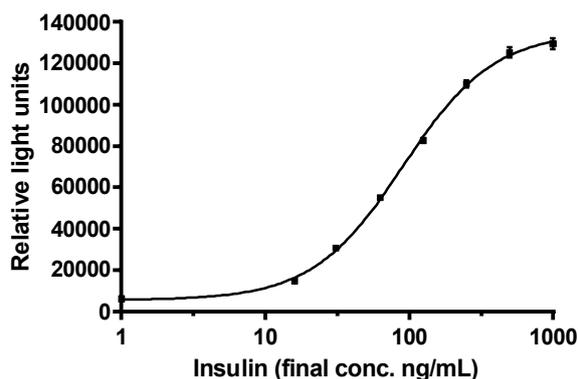


Figure 1. Example of Insulin calibration curve.

| Calibrator | Insulin |
|------------|---|
| | Suggested calibrator solution conc. (ng/ml) |
| A | 2000 |
| B | 1000 |
| C | 500 |
| D | 250 |
| E | 125 |
| F | 63 |
| G | 31 |
| H | 0 |

Table 1. Suggested calibrator concentrations for Insulin.

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 concentration** of 0-1000 ng/mL
3. Add 40 μ L calibrator, control and sample solution in duplicates to the assigned wells (final concentration will be half of solution concentration).
4. Thaw a vial of *iLite*[®] Insulin Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully ten times with pipette in order to ensure a homogeneous distribution of cells.
5. Dilute 250 μ L cell suspension with 5.75 mL diluent.
6. Add 40 μ L 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

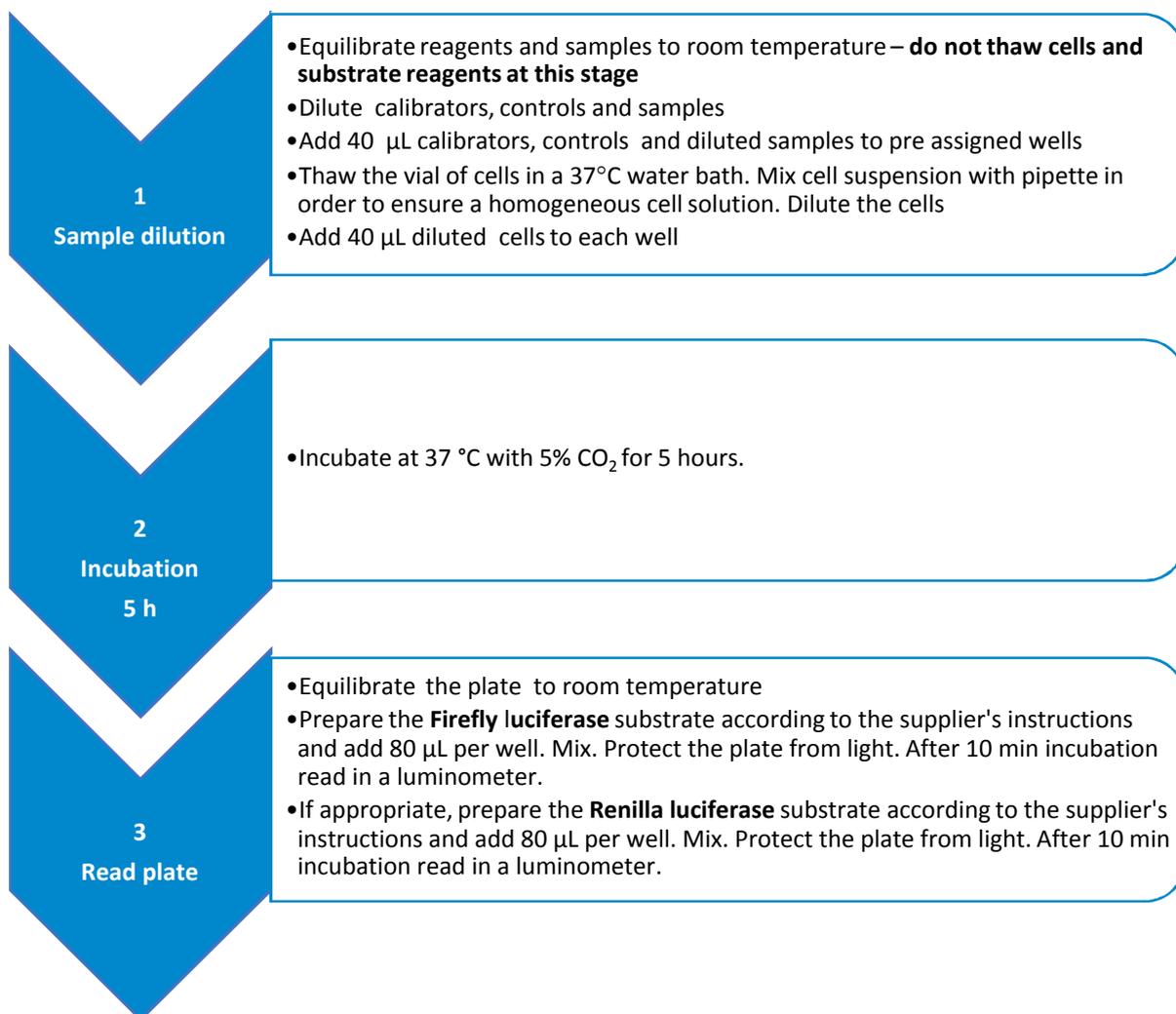
8. Equilibrate the plate and the substrate solutions to room temperature.
9. Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 μ L per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.
10.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

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**Quantification of functional Insulin using *iLite*[®] Insulin Assay Ready Cells****Troubleshooting and FAQ**

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

References

1. American Society of Health-System Pharmacists (2009). *Insulin Injection*. PubMed Health. National Center for Biotechnology Information, U.S. National Library of Medicine. Retrieved 2012-10-12.