Determination of anti-insulin neutralizing antibodies using *iLite™* Insulin Assay Ready Cells

This application note contains a suggested protocol and performance data. Each individual laboratory must set up their own method and perform relevant validations.
For research and professional use only.

Background

Insulin is a peptide hormone produced by the beta cells of the pancreas. Its main function is to enhance the uptake of glucose by target cells and thereby regulate the metabolism of carbohydrates, fats and proteins. Release of insulin into the circulation is dependent on the blood glucose levels. The effect of insulin can be hampered either by direct destruction of the beta cells (Type I diabetes) or decreasing the ability of the target cells to uptake glucose (Type II diabetes). In both forms of diabetes, administration of exogenous insulin is an essential part of the treatment. However, prolonged therapies with drugs such as insulin may lead to development of neutralizing antibodies counteracting the physiological effect.

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 functional activity of insulin in the sample. In the presence of neutralizing antibodies against insulin, the amount of free insulin is reduced, resulting in a decreased stimulation of Firefly luciferase production. The Firefly luciferase signal is thus inversely proportional to the amount of neutralizing antibodies in a sample. The iLite Insulin Assay Ready Cells can therefore be utilized as a highly sensitive assay for determination of anti-insulin neutralizing antibodies in test samples including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite™ Insulin Assay Ready Cells	Euro Diagnostica	BM3060
Diluent (RPMI 1640 with GlutaMAX™, containing 10% FBS and 1% Penicillin-Streptomycin).	Gibco	61870-044 (RPMI 1640)26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Anti-Insulin antibody	Abcam	Ab7842
Insulin or analogues	Life Technologies	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 Euro Diagnostica for list of recommended suppliers	NA

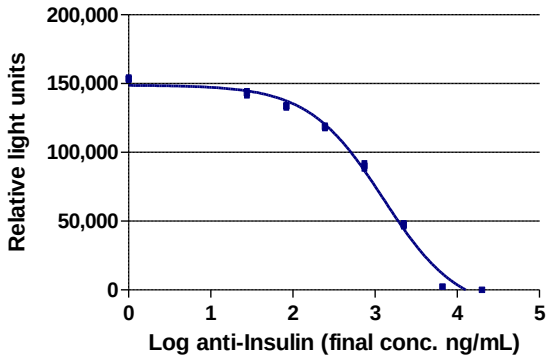
Incubator, 37 °C with 5% CO_2	NA	NA
Water bath, 37 °C	NA	NA
Single-channel and multi-channel pipettes with polypropylene disposable tips	NA	NA
Polypropylene tubes or plate for dilution	NA	NA
Single-use polypropylene reservoir	NA	NA
Plate shaker	NA	NA
Timer	NA	NA

Protocol

Preparation of anti-Insulin neutralizing antibody

Anti-insulin antibody from Abcam has successfully been used to neutralize insulin and inhibit the insulin regulated Firefly luciferase expression in iLite Insulin Assay Ready Cells (refer to the table and graph below).

Final Insulin conc. 500 ng/mL	**Anti-Insulin antibody** Suggested solution concentrations, ng/mL
A	80 000
B	26 666
C	8 888
D	2 963
E	988
F	329
G	110
H	0



Incubation

1. Design a plate layout. It is recommended to perform test at least in duplicate.
2. Perform a serial dilution of the reference anti-insulin antibody. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference anti-insulin antibody dilutions, controls and samples to assigned wells.
4. Add 20 µL of 2 µg/mL insulin to all wells.
5. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37 °C with 5% CO_2.
6. Thaw the 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 uniform solution of cells.
7. Dilute 250 µL cells with 5.75 mL Diluent
8. Add 40 µL diluted cells to each well.
9. Place the lid on the plate, mix and incubate for 5 hours at 37 °C with 5% CO_2.

Adding substrate solutions

1. Equilibrate the plate and the substrate solutions to room temperature.

2. Prepare the **Firefly luciferase** substrate according to the suppliers instructions and add 80 µL per well. Mix and protect the plate from light. Read in a luminometer after 10 minutes incubation at room temperature.
3. If appropriate, prepare the **Renilla luciferase** substrate according to the suppliers instructions and add 80 µL per well. Mix and protect the plate from light. Read in a luminometer after 20 minutes incubation at room temperature.

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 suppliers/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. Biomonitor *iLite™* cell-based products are covered by patents which are the property of Euro Diagnostica AB and any attempt to reproduce the delivered *iLite™* Assay Ready Cells is an infringement of these patents.

Quick Guide – Quantification of Insulin neutralizing antibodies using *iLite™* Insulin Assay Ready Cells

1 Sample dilution

- Equilibrate reagents and samples to room temperature – **do not thaw cells and substrate reagents at this stage**
- Serial dilute reference anti-insulin antibody
- Add 20 µL of ref. anti-insulin antibody solutions, controls and samples to pre-assigned wells
- Add 20 µL of insulin

2 Incubation 30 minutes

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

3 Add cells

- Thaw the vial of cells in a 37°C water bath. Mix cell suspension with pipette in order to ensure a uniform cell suspension. Dilute the cells
- Add 40 µL diluted cells to each well

4 Incubation 6 hours

- Incubate at 37°C with 5% CO2 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. Read in a luminometer after 10 min incubation.
- 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. Read in a luminometer after 20 min incubation.

Troubleshooting and FAQ

Please consult Euro Diagnostica's website at www.eurodiagnostica.com.

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