Quantification of hCG inhibitor using *iLite*® hCG 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

Human chorionic gonadotropin (hCG) is a heterodimeric glycoprotein hormone. The larger beta subunit is specific for hCG while the smaller alpha subunit is identical to the alpha subunit of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH). Both hCG and LH binds to the common G-protein-coupled transmembrane Lutropin-choriogonadotropin hormone receptor (LHCGR), present mainly in ovary and testis, but also in uterus and breast (1).
In men and non-pregnant women, hCG levels are low, but at the beginning of pregnancy, hCG is upregulated which stimulates the production of progesterone in the corpus luteum. In addition, hCG has been found to be expressed by several tumors, influencing tumor formation and metastatic growth, and has therefore been suggested as a prognostic biomarker in certain malignancies (2).
Clinically, gonadotropins are part of fertility treatments, hCG is provided under several brand names ex., Pregnyl, Ovitrelle and Menopur. hCG is either extracted from the urine of pregnant women or produced using recombinant DNA technology (3).
The assessment of factors influencing the hCG - LHCGR-receptor interaction such as neutralizing antibodies or other antagonists is of high importance (4,5). The *iLite*® platform offers a cell-based assay that enables the study of hCG, its receptor and their interaction.

Principle of the assay

The *iLite*® hCG Assay Ready Cells are engineered cells, optimized to express Firefly luciferase (FL) under the control of an hCG responsive promoter. Binding of hCG to the Lutropin-choriogonadotropic hormone receptor (LHCGR) results in activation of the hCG regulated Firefly luciferase reporter gene construct. *iLite*® hCG 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 hCG induced FL activity, and renders assay results independent of variations in cell number or serum matrix effects *(there is a slight dose dependency trend for the normalization signals and a shift upwards can be detected in higher hCG concentrations – see section "Normalization")*. 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 hCG in the sample. In the presence of inhibitory activity against hCG, the amount of free hCG 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 hCG in a sample. The *iLite*® hCG Assay Ready Cells can therefore be utilized as an assay for quantification of hCG inhibitor activity in test samples, including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® hCG Assay Ready Cells	Svar Life Science	BM4080
Diluent (DMEM containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin).	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Monoclonal anti-human chorionic gonadotropin antibody	Thermofisher Scientific	MIH9801
hCG or analogues	R&D Systems	7727-CG/CF
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 hCG inhibitor

hCG inhibitor from Thermofisher Scientific has successfully been used to neutralize hCG and inhibit the hCG regulated Firefly luciferase expression in *iLite*® hCG Assay Ready Cells (refer to the table and graph below)

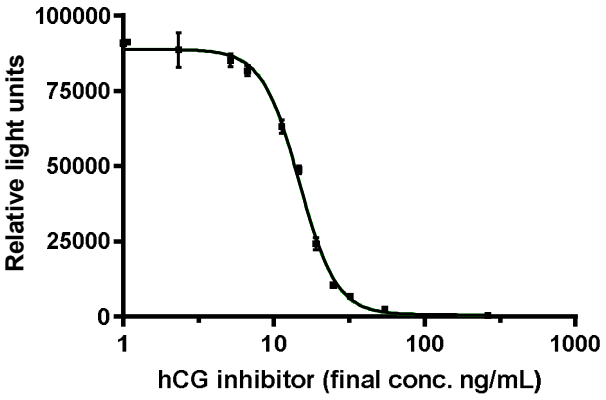

Figure 1. Example of hCG inhibitory curve

Final 3.1 ng/mL hCG	MAb anti-hCG Suggested calibrator solution concentrations, ng/mL
1	1 050
2	218
3	129
4	99
5	76
6	59
7	45
8	27
9	21
10	9.3
11	4.3
12	0

Table 1. Suggested calibrator solution concentrations for hCG inhibitor

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicates.
2. Perform a serial dilution of the reference hCG inhibitor. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference hCG inhibitor dilutions, controls and samples to assigned wells (final concentration will be a quarter of solution concentration).
4. Add 20 µL of 12.5 ng/ml hCG to all wells (final concentration will be 3.1 ng/mL hCG).
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*® hCG 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.
7. Dilute 250 µL cell suspension with 5.75 mL Diluent.
8. Add 40 µL diluted cells to each well - **Note:** Delayed addition of the diluted cell suspension to the plate might cause a shift in EC50 value.
9. Place the lid on the plate, mix and incubate for 3 hours at 37 °C with 5% CO_2.

Adding substrate solutions

10. Equilibrate the plate and the substrate solution to room temperature.
11. 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.
12. 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.

Normalization

The reporter gene used for normalization, Renilla luciferase, is under the control of a tyrosine kinase promoter, and is thus constitutively expressed. Unspecific effects such as serum matrix effects or differences in cell number can be obviated by relating the specific Firefly signal with the Renilla normalization signal through simple division.

In the case of growth factors such as hCG, high concentrations can result in a quantifiable effect on the general machinery of the cell, such as the transcription rate of polymerases or the activity of certain elongation factors. This highly reproducible effect is seen as an increase in the normalization gene readout, proportional to the increase of hCG concentration (see Figure 2 below). Normalization against the Renilla signal will compensate the effects of hCG on the cellular machinery as well as non-specific effects such as serum matrix effects or differences in cell number, the result can be seen below.

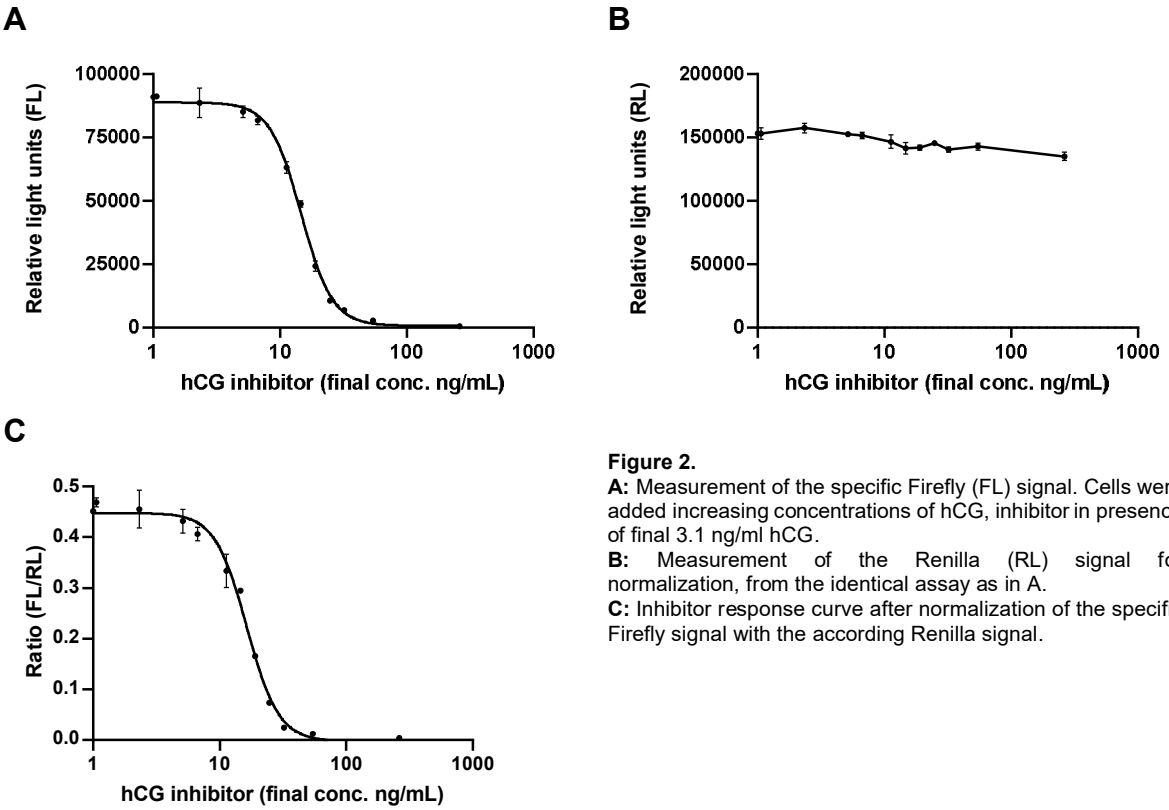

Figure 2.
A: Measurement of the specific Firefly (FL) signal. Cells were added increasing concentrations of hCG, inhibitor in presence of final 3.1 ng/ml hCG.
B: Measurement of the Renilla (RL) signal for normalization, from the identical assay as in A.
C: Inhibitor response curve after normalization of the specific Firefly signal with the according Renilla signal.

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.

QUICK GUIDE

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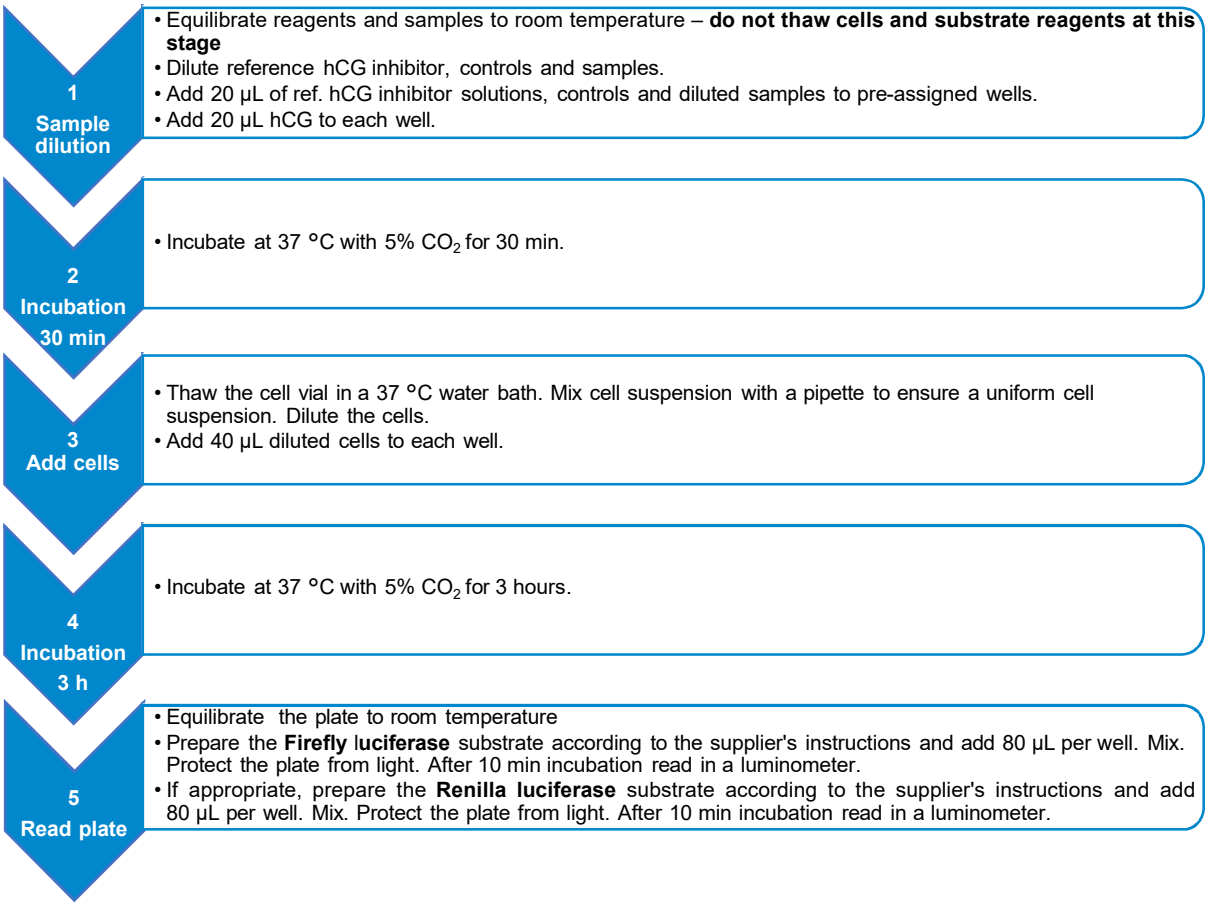

Troubleshooting and FAQ

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

References

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4. Morte C et al. *Assessment of the immunogenicity of gonadotrophins during controlled ovarian stimulation.* Am J Reprod Immunol. 2017 Sep;78(3).
5. Kara E et al. *Modulation of Gonadotropins Activity by Antibodies.* Front Endocrinol (Lausanne). 2019 Feb 18;10:15.