# Quantification of functional IL-12 using *iLite*® IL-12 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

Interleukin-12 (IL-12) is a 70 kDa cytokine mainly produced by macrophages, neutrophils and dendritic cells in response to stimulation by inflammatory antigens. Stimulation of T-cells, involvement in T-cell differentiation of Th1 cells and stimulation of IFN-gamma and TNF-alpha production are key functions of IL-12. IL-12 is composed of two subunits, p35 and p40, covalently linked by a single disulfide bond. The p40 subunit, which binds to the receptor chain IL-12Rβ1, is shared with another heterodimeric cytokine, IL-23. However, the two cytokines expert distinct non-redundant biological functions (1). Therapeutic agents targeting both IL-12 and IL-23 cytokines are currently used to treat psoriasis and psoriatic arthritis, and related agents are in clinical testing for a variety of inflammatory disorders (2).

## Principle of the assay

The *iLite*® IL-12 Assay Ready Cells are engineered cells, optimized to express Firefly luciferase (FL) under the control of an IL-12 responsive promoter. Binding of IL-12 to the IL-12 receptor (IL12R1 and IL12R2) results in activation of the IL-12 regulated Firefly luciferase reporter gene construct. *iLite*® IL-12 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 IL-12 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 IL-12 in a sample (Fig.1).

## Specimen collection

The *iLite*® IL-12 Assay Ready Cells can be used for measuring concentration of IL-12 in test samples including human serum.

## Material and equipment needed

| Material and equipment | Suggested supplier | Reference |
|---|---|---|
| *iLite*® IL-12 Assay Ready Cells | Svar Life Science | BM4012 |
| Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin) | Gibco | 61870-044 (RPMI)<br>26140-079 (FBS)<br>15140-122 (Penicillin-Streptomycin) |
| IL-12 or analogues | R&D Systems | 219-IL |
| 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 | NA | NA |
| Timer | NA | NA |

## Protocol

### Preparation of calibrators (IL-12)

Recombinant IL-12 from R&D Systems has successfully been used to stimulate the *iLite*® IL-12 Assay Ready Cells. The below table shows the dilutions of IL-12, used for QC release of the *iLite*® IL-12 Assay Ready Cells.

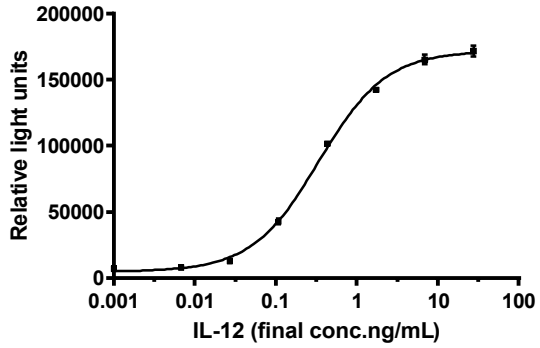


**Figure 1**. Example of IL-12 calibration curve.

| Calibrator | IL-12 Suggested calibrator solution conc. (ng/ml) |
|---|---|
| **A** | 56 |
| **B** | 14 |
| **C** | 3.5 |
| **D** | 0.87 |
| **E** | 0.22 |
| **F** | 0.054 |
| **G** | 0.014 |
| **H** | 0 |

**Table 1.** Suggested calibrator **solution concentrations** for IL-12.

### 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 0-28 ng/mL.
3. Add 40 µL calibrators, controls and samples in duplicate to assigned wells (final concentration will be half of the solution concentration).
4. Thaw a vial of *iLite*® IL-12 Assay Ready Cells in a water bath at 37°C with gentle agitation. The cell suspension is mixed very carefully ten times with a 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% $CO_2$.

### 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 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

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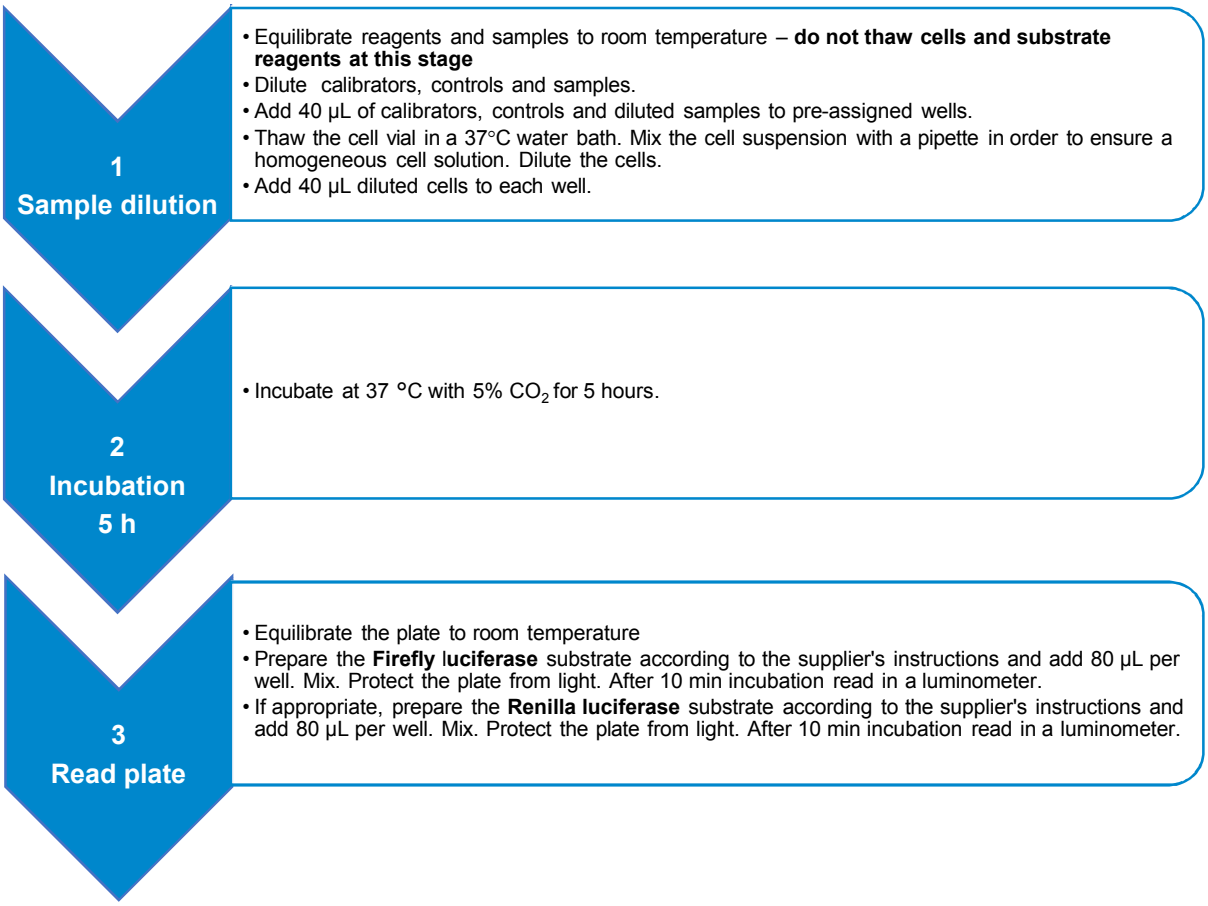


## Troubleshooting and FAQ

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

## References

1. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. (April 1993). *Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages.* Science 260: 547–5499 (1993).
2. Teng MW, Bowman EP, McElwee JJ, Smyth MJ, Casanova JL, Cooper AM, Cua DJ. *IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases.* Nature Medicine 21: 719–729 (2015).

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