Quantification of IL-12 inhibitor activity using *iLite*™ IL-12 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

Interleukin 12 (IL-12) is a 70 kDa cytokine produced principally by macrophages, neutrophils and dendritic cells in response to stimulation by antigen. IL-12 is a T cell-stimulating factor, playing a critical role in the regulation of T-helper 1 cell responses. 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 under the control of an IL-12 responsive promoter. When IL-12 binds to the hetero-dimeric cell surface receptor composed of IL-12Rβ1 and IL-12Rβ2 it activates the IL-12 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 IL-12 in the sample. In the presence of inhibitory activity against IL-12, the amount of free IL-12 is reduced, resulting in a decreased stimulation of Firefly luciferase production. The Firefly luciferase signal is inversely proportional to the amount of inhibitory activity in a sample. The *iLite*™ IL-12 Assay Ready Cells can therefore be utilized as a highly sensitive assay for quantification of IL-12 inhibitor activity in test samples, including human serum.

Material and equipment needed

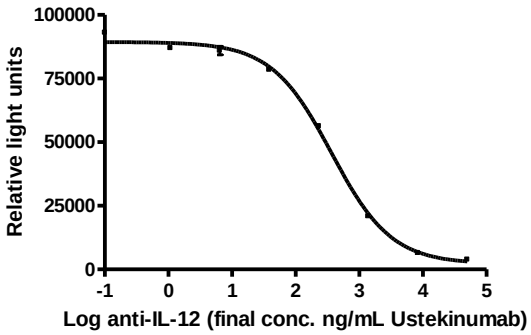
Material and equipment	Suggested supplier	Reference
iLite™ IL-12 Assay Ready Cells	Euro Diagnostica	BM4012
Diluent (RPMI 1640 with GlutaMAX™, containing 10% FBS and 1% Penicillin-Streptomycin).	Gibco	61870-044 (RPMI 1640) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Ustekinumab or analogues	NA	NA
IL-12 or analogues	R&D	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 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 IL-12 inhibitor

Ustekinumab has successfully been used to inhibit IL-12 and the IL-12 regulated Firefly luciferase expression in *iLite*™ IL-12 Assay Ready Cells (refer to the table and graph below).

Final IL-12 conc. 6 ng/mL	**Ustekinumab** Suggested solution concentrations, ng/mL
A	200 000
B	33 332
C	5 556
D	924
E	156
F	25.6
G	4.4
H	0



Incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicate.
2. Perform a serial dilution of the reference IL-12 inhibitor. Ensure matrix consistency between reference inhibitor solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference IL-12 inhibitor dilutions, controls and samples to assigned wells.
4. Add 20 µL of 24 ng/mL IL-12 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*™ IL-12 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 supplier's 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 supplier's 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 IL-12 inhibitor activity using iLite™ IL-12 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 IL-12 inhibitor
- Add 20 µL of reference IL-12 inhibitor solutions, controls and samples to pre-assigned wells
- Add 20 µL of IL-12 to each well

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 a pipette in order to ensure a uniform cell suspension. Dilute the cells
- Add 40 µL diluted cells to each well

4 Incubation 5 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 www.eurodiagnostica.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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