# Quantification of functional VEGF using *iLite™* VEGF 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

Vascular Endothelial Growth Factor (VEGF) is a signaling protein which is involved in both normal vascular growth and pathological angiogenesis. Without angiogenesis, growth of solid tumors would be limited by oxygen and nutrient supply. Tumors which express VEGF can overcome this limitation and are thus able to grow and metastasize. For this reason, different anti-cancer therapies targeting VEGF have emerged, e.g. a humanized anti-VEGF antibody bevacizumab (Avastin™, Genentech) is currently widely used as a first-line therapy for colorectal cancer (1,2).

## Principle of the assay

The *iLite™* VEGF Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of a VEGF responsive promoter. Binding of VEGF to the VEGF receptor 2 (VEGFR2) results in activation of the VEGF 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 functional VEGF in the sample (Fig.1).

## Specimen collection

The *iLite™* VEGF Assay Ready Cells can be used for measuring concentration of VEGF in test samples including human serum.

## Material and equipment needed

| Material and equipment | Suggested supplier | Reference |
|---|---|---|
| *iLite™* VEGF Assay Ready Cells | Euro Diagnostica | BM4020 |
| Diluent (DMEM + 9% heat inactivated FBS + 1% Penicillin Streptomycin) | Gibco | 31966-021 (DMEM)<br>26140-079 (FBS)<br>15140-122 (Penicillin-Streptomycin) |
| VEGF or analogues | Gibco | PHC9391 |
| 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 |

## Preparation of calibrators (VEGF)

VEGF from Gibco have successfully been used to stimulate the iLite VEGF Assay Ready Cells. The below table shows the dilutions of VEGF, used for QC release of the iLite VEGF Assay Ready Cells.

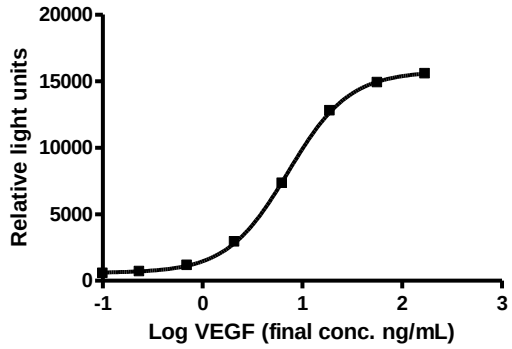



Figure 1. Example of VEGF calibration curve

| Calibrator | VEGF |
|---|---|
| | Calibrator conc. (ng/mL) |
| A | 170 |
| B | 57 |
| C | 19 |
| D | 6.3 |
| E | 2.1 |
| F | 0.70 |
| G | 0.23 |
| H | 0 |

Table 1. Suggested calibrator concentrations for VEGF

# Protocol

## Incubation

1. Design a plate layout.
2. Dilute calibrators, controls and samples to fall within the expected assay values of 0-170 ng/mL.
3. Add 40 µL calibrators, controls and samples in duplicate to assigned wells.
4. Thaw the vial of *iLite™* VEGF 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.
5. Dilute 2 times 125 µL cells 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 18 hours at 37 °C with 5% $CO_2$.

## Adding substrate solutions

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

9. 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.

10. 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.

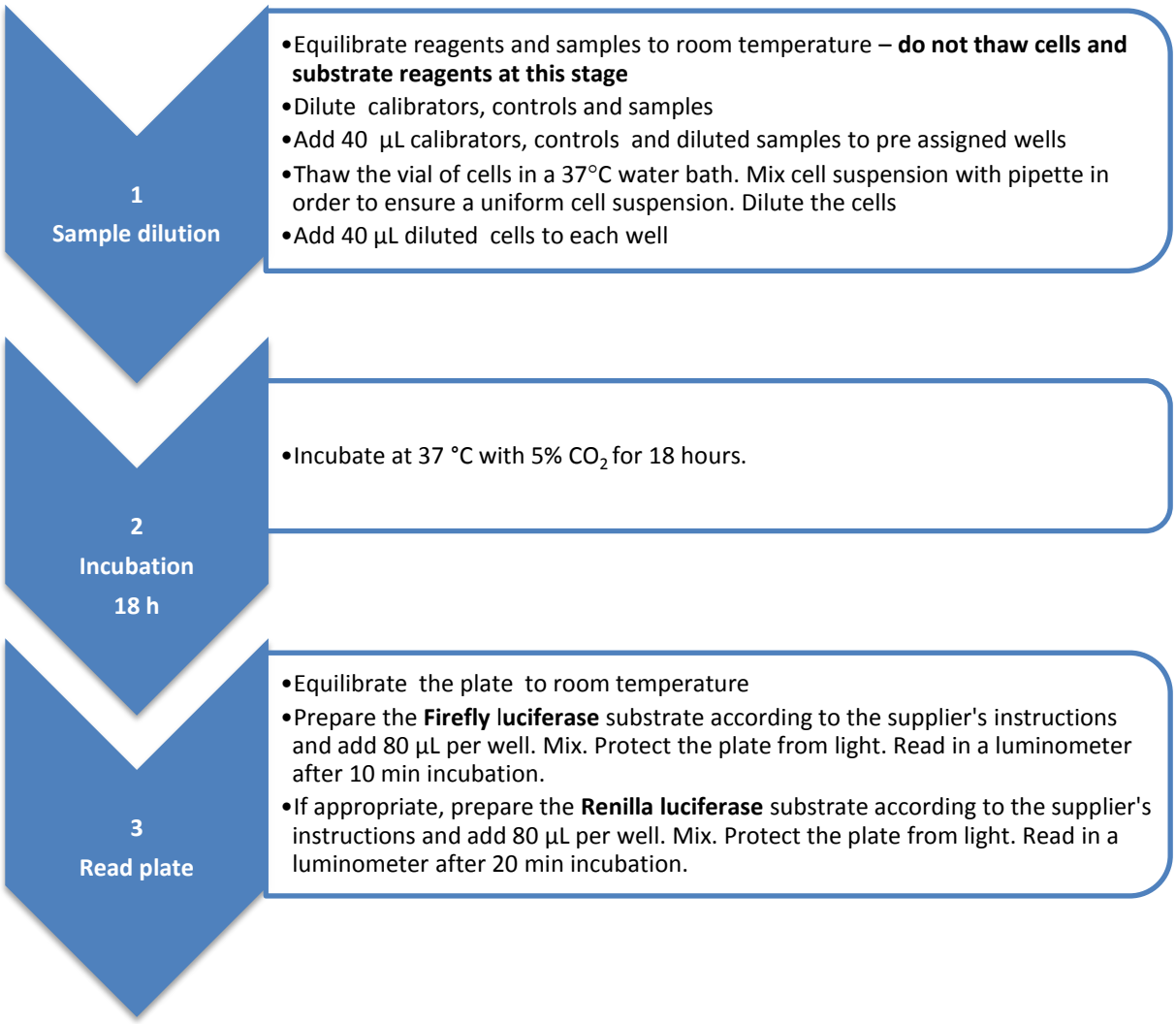
## Precautions

- 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 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. 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 functional VEGF using *iLite*™ VEGF Assay Ready Cells




## Troubleshooting and FAQ

Please consult Euro Diagnostica's website.

## References

1. Wang Y, Fei D, Vanderlaan M, Song A. *Biological activity of bevacizumab, a humanized anti-VEGF antibody in vitro.* Angiogenesis 7:335-345 (2004).

2. Risau, W. *Mechanisms of angiogenesis.* Nature 386: 671 – 674 (1997).



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