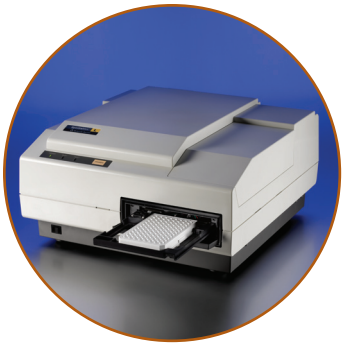
Molecular Devices

Chemiluminescent VEGF ELISA Using the SpectraMax L Microplate Luminometer

SPECTRAMAX APPLICATION NOTE #9



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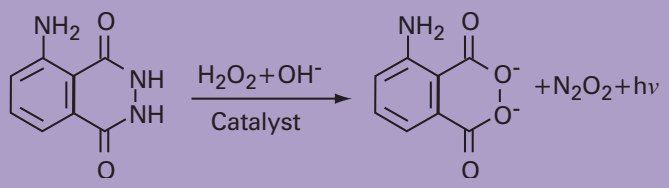
INTRODUCTION

Vascular endothelial growth factors (VEGFs) are a family of secreted polypeptides that have been implicated in mammalian vascular development and in disease processes involving abnormal blood vessel growth. VEGFs are expressed during embryogenesis, where deletion of even a single VEGF allele severely disrupts vasculogenesis and is embryonic lethal. $VEGF_{165}$ is the most abundant and biologically active isoform of VEGF found in mammals.[1] The QuantiGlo Chemiluminescent VEGF Immunoassay is a solid phase ELISA that measures $VEGF_{165}$ levels in cell culture supernatants, serum, plasma, saliva, and urine. It uses the quantitative sandwich enzyme immunoassay technique in which a monoclonal antibody specific for VEGF is coated onto a microplate and standards and samples are added to the wells.[2] Unbound material is washed away, and a horseradish peroxidase-linked polyclonal antibody-specific for VEGF is added to the wells. An additional series of washes removes unbound antibody-enzyme reagent, then an enhanced luminol/peroxide substrate is added to the wells. (See Figure 1.) Light produced in proportion to the amount of VEGF initially bound to the wells is then measured using a microplate luminometer.

Oxidation of Luminol (Figure 1)



An enhanced luminol/peroxide substrate is oxidized, resulting in emission of light.

Assay results were detected using the SpectraMax® L microplate luminometer. This instrument is suitable for both flash and glow applications and is compatible with 96- and 384-well microplate formats. Its dedicated luminescence optical design yields an extremely high signal-to-noise ratio and extremely low crosstalk, with a dynamic range of more than eight orders of magnitude. Similar results may be obtained using the luminescence mode of SpectraMax® M5/M5^e and FlexStation® 3 multi-mode microplate readers. Data collection and calculations were performed using SoftMax® Pro software, which includes preconfigured protocols to simplify data acquisition and analysis.

MATERIALS

- QuantiGlo Human VEGF Immunoassay (R&D Systems P/N QVE00B). This kit contains all of the reagents and materials required to perform the assay, including assay standards and a microplate pre-coated with a monoclonal antibody specific for VEGF.
- SpectraMax L microplate luminometer. Note: this assay can also be detected using the luminescence mode of SpectraMax M5/M5^e and FlexStation 3 multi-mode microplate readers.

METHODS

Preparation of reagents and standards

Step 1. All reagents were brought to room temperature before use.

Step 2. Wash Buffer Concentrate was diluted 10-fold with deionized water.

Step 3. Working Glo Reagent was prepared 15 minutes to 4 hours before use by combining 1 part Glo Reagent A and 2 parts Glo Reagent B in a capped plastic tube, protected from light.

Step 4. The VEGF standard was reconstituted with 0.5 mL deionized water and allowed to sit for a minimum of 15 minutes, with gentle agitation prior to making dilutions. Working standards were prepared by making a serial 1:10 dilution of the stock standard in

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Calibrator Diluent RD5L. Concentrations of working standards ranged from 20,000 to 1.3 pg/mL.

Assay procedure

Step 1. 150 µL of Assay Diluent RD1-8 was pipetted into each well.

Step 2. 50 µL of working standard or blank (Calibrator Diluent) was pipetted into triplicate wells of the supplied microplate. The microplate was incubated for 2 hours at room temperature with shaking (500 ± 50 rpm recommended).

Step 3. Wells were aspirated and washed 4 times with 400 µL Wash Buffer using a multichannel pipettor. After the last wash, the remaining Wash Buffer was aspirated and the plate was blotted against clean paper towels.

Step 4. 200 µL of VEGF Conjugate was added to each well, and a new adhesive strip was applied. The microplate was incubated for 3 hours at room temperature, with shaking as in step 2.

Step 5. Aspiration and washing steps were repeated as in step 3.

Step 6. 100 µL Working Glo Reagent was added to each well. The microplate was incubated for 5-20 minutes at room temperature, protected from light.

Step 7. RLU values were determined using the SpectraMax L microplate luminometer. Instrument settings included the following: 1 second integration time, AutoRange PMT setting, and 470 nm target calibration wavelength.

RESULTS

Figure 1 shows the VEGF standard curve obtained with the SpectraMax L microplate luminometer using the settings outlined above. The dynamic range for this assay spans greater than four orders of magnitude, with a calculated sensitivity of about 1.5 pg/mL, based on a concentration giving a signal 3 times the standard deviation of the background. This correlated well with R&D Systems' claim of a mean minimum detectable dose of 3.3 pg/mL for the kit. The SpectraMax M5 and FlexStation 3 multi-mode microplate readers yielded similar results (data not shown). Results were analyzed and the standard curve was plotted using SoftMax Pro software.

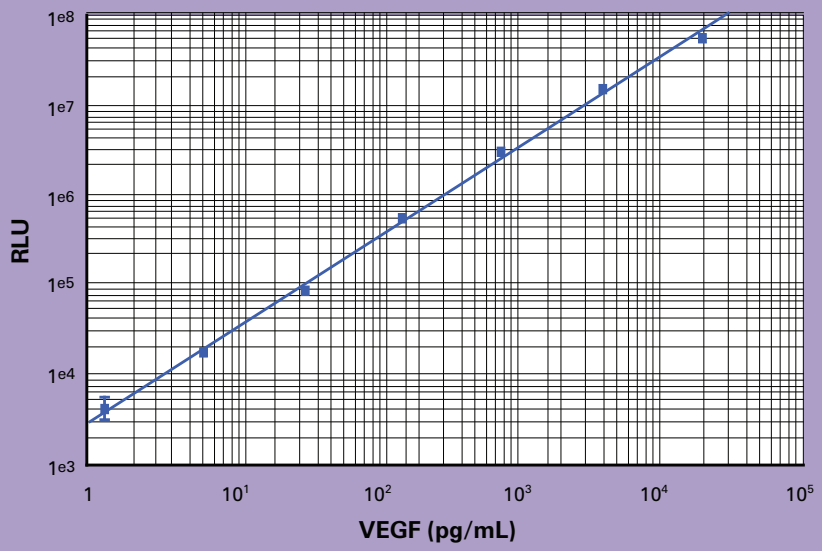
SUMMARY

The Quantiglo Human VEGF Immunoassay is a highly sensitive method for assaying VEGF in a variety of sample types. The SpectraMax L microplate luminometer with SoftMax Pro software provides excellent sensitivity and dynamic range for this assay as well as simplified data acquisition and analysis.

REFERENCES

1. Holmes, David IR and Zachary, Ian (2005). The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. *Genome Biology* 6: 209.
2. Quantiglo Human VEGF Immunoassay (P/N QVE00B) package insert.

VEGF Standard Curve (Figure 2)



Standards ranging from 20,000 to 1.3 pg/mL were assayed as described in the assay procedure. The microplate was read on a SpectraMax L microplate luminometer with a 1-second intergration time and target calibration wavelength of 470 nm. Results were plotted using SoftMax Pro software.

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