The ELISAONE™ assay performed on a multi-mode microplate reader from BMG LABTECH

Miriam Uppill, Antony Sheehan and Ron Osmond
TGR BioSciences Pty Ltd.

- ELISAONE™ is a new technology from TGR BioSciences to measure ELISA in fluorescence mode
- ELISAONE™ is both robust and sensitive as evidenced by the data presented for the detection of EGF, TNFα and IL-2.

Introduction

ELISAONE™ technology has been developed by TGR BioSciences, to provide a means of running high-performance sandwich immunoassays in a user-friendly 96-well format. ELISAONE™ assays use a traditional immuno-sandwich format, but with a major difference. The analyte and both antibodies are added to the ELISAONE™ assay microplate at the same time, allowing solution-phase binding. After a short incubation period, unbound assay reagents and analytes are washed away, and only immunocomplexes containing both antibodies are detected. The whole process can take as little as 60 minutes to complete, and requires just a single wash step. In contrast to other ELISA formats, the target-specific antibodies needed for the assay are not pre-bound to the microplate itself. The binding of antibodies to the analyte takes place in solution, allowing for efficient binding. This not only reduces assay times, but also affords several other benefits to the user. As the antibodies are not fixed to the plate, assays for several different targets can be performed in different wells on the same microplate, side-by-side. Another important benefit is reagent lifetime – many ELISA kits need to be discarded one month after opening, largely because the antibody-coated plates are often not stable after opening. With ELISAONE™ however, the expensive assay reagents are in reusable bottles, and last a long time after the ELISAONE™ microplate has expired. The inexpensive microplate can simply be discarded and another ordered, as they’re all identical.

ELISAONE™ assays for EGF, TNFα and IL-2 developed by TGR BioSciences, were used here to demonstrate the robustness and high sensitivity of the ELISAONE™ technology.

Assay Principle

**ELISAONE™ – Protocol Overview**

![ELISAONE™ protocol overview](image)

Fig. 1: ELISAONE™ assay principle.

Materials & Methods

- Human EGF, IL-2 and TNFα were all from R&D Systems

Results & Discussion

1. Assay reproducibility and sensitivity

![ELISAONE™ assay sensitivity and reproducibility](image)

Fig. 2: ELISAONE™ readouts depending on either EGF concentration (A) or IL-2 concentrations (B) measured at three different days.

Keywords: EGF, ELISAONE, IL-2, Immunoassay, TNFα
Three assays were assessed for reproducibility over 3 separate experiments. Each day, either recombinant human EGF (Fig 2A), recombinant human IL-2 (Fig. 2B) or recombinant human TNF\(\alpha\) (data not shown) were diluted to various concentrations in PBS containing 0.5% BSA in 96-well ELISAONE assay plates (n=4 wells/concentration point). ELISAONE was added to the wells, and the assays were incubated for one hour, washed, and incubated with ELISAONE Substrate Mix for 10 min. The plates were read immediately using a POLARstar Omega microplate reader. The limit of detection (LOD) was approximated using the signal obtained with buffer-only controls + 3x standard deviations.

### Conclusion

This application note establishes ELISAONE™ [TGR BioSciences: www.tgrbio.com] as a robust and sensitive technology for the detection of biomarkers in biological samples. Its ease of implementation and substantially reduced preparation time, as compared to traditional ELISA assays, represents a substantial advance in ELISA technology. The filter-based microplate reader from BMG LABTECH provides an easy-to-use instrument that will measure this fluorescence based assay quickly, accurately and robustly.

<table>
<thead>
<tr>
<th>LOD (EGF)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 pg/mL</td>
<td>0.4 pg/mL</td>
<td>0.1 pg/mL</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LOD (IL-2)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pg/mL</td>
<td>1 pg/mL</td>
<td>1 pg/mL</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LOD (TNF(\alpha))</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 pg/mL</td>
<td>1 pg/mL</td>
<td>1 pg/mL</td>
<td></td>
</tr>
</tbody>
</table>

### Analyte recovery from tissue-culture supernatants

The assays ability to recover known amounts of analyte from tissue culture media was investigated.

**Fig. 3:** Analyte recovery measurements for EGF (A) and TNF\(\alpha\) (B).

Each day, either recombinant human EGF (Fig. 3A), recombinant human IL-2 (data not shown) or recombinant human TNF\(\alpha\) (Fig. 3B) were diluted to concentrations representing more than a 3-Log range (20 pg/mL, 100 pg/mL, 500 pg/mL or 2500 pg/mL) in RPMI containing 10% FBS in 96-well ELISAONE assay plates (n=2 wells/concentration point). ELISAONE antibody mix was added to the wells, and the assays were incubated for one hour, washed, and incubated with ELISAONE Substrate Mix for 10 min. The plates were read immediately. The assay was able to accurately recover analytes over a range of 3-logs difference in concentration.

The recovery was determined using an analyte-specific standard curve, diluted in PBS containing 10% FBS, and fitted to a non-linear 4-parameter sigmoidal curve. These calculations could be easily performed using the MARS evaluation software that interfaces with all BMG LABTECH microplate readers.