The ELISA*ONE*™ assay performed on a multi-mode microplate reader from BMG LABTECH

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

Introduction

ELISA*ONE*™ technology has been developed by TGR BioSciences, to provide a means of running high performance sandwich immunoassays in a user-friendly 96-well format. ELISA*ONE* assays use a traditional immuno-sandwich format, but with a major difference. The analyte and both antibodies are added to the ELISA*ONE* 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 ELISA*ONE* however, the expensive assay reagents are in reusable bottles, and last a long time after the ELISA*ONE* microplate has expired. The inexpensive microplate can simply be discarded and another ordered, as they're all identical.

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

Assay Principle

ELISA*ONE*™ - Protocol Overview

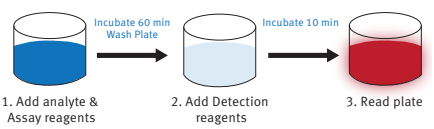

Fig. 1: ELISA*ONE* assay principle.

Materials & Methods

- Human EGF, IL-2 and TNFα were all from R&D Systems
- ELISA*ONE*™ microplates and assay reagents (Antibody Mix, Wash Solution and Substrate Mix) were from TGR BioSciences
- Filter-based microplate reader was from BMG LABTECH

Method:

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

Briefly, analyte (50 µL) prepared in either BSA/PBS or RPMI containing 10% FBS depending on the experiment, was dispensed into duplicate wells of an ELISA*ONE* microplate. Antibody Mix (50 µL) – containing 2 antibodies specific for the target analyte – was added to the wells immediately after the analyte. The wells were sealed with a plate seal, and the ELISA*ONE* microplates were incubated for 1 hr at room temp, with gentle shaking. During this time the antibodies specifically bind to the analyte. Unbound components were aspirated from the wells, and the wells were washed manually with 3 x 200 µL of 1X Wash Solution. Substrate Mix (50 µL) was added to the wells, the microplate was covered with foil, and incubated for 10 min at room temp, with gentle shaking. The plates were uncovered, and the fluorescence signal (Ex 540 nm and Em 590 nm) was measured.

For recovery experiments, the amount of analyte in the well was calculated from a standard curve run on the same plate, prepared from 10-fold dilutions of analyte ranging from 1000 pg/mL to 0.1 pg/mL for EGF, and 10 ng/mL to 0.1 pg/mL for IL-2 and TNFα. The standard curve was fitted to a 4-parameter sigmoidal dose response equation, and the fitted equations were used to estimate the amounts of analyte recovered in each experiment.

Results & Discussion

1. Assay reproducibility and sensitivity

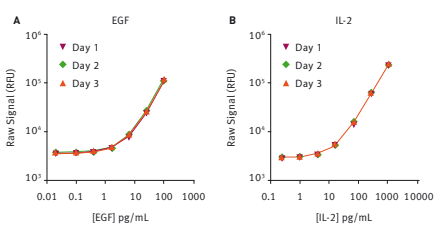

Fig. 2: ELISA*ONE* readouts depending on either EGF concentration (A) or IL-2 concentrations (B) measured at three different days.

Rev. 03/2011
Keywords: **EGF, ELISA*ONE*, 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 TNFα (data not shown) were diluted to various concentrations in PBS containing 0.5% BSA in 96-well ELISA*ONE* assay plates (n=4 wells/concentration point). ELISA*ONE* was added to the wells, and the assays were incubated for one hour, washed, and incubated with ELISA*ONE* 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.

	Day 1	Day 2	Day 3
LOD (EGF)	0.1 pg/mL	0.4 pg/mL	0.4 pg/mL
LOD (IL-2)	1 pg/mL	4 pg/mL	1 pg/mL
LOD (TNFα)	4 pg/mL	1 pg/mL	1 pg/mL

2. Analyte recovery from tissue-culture supernatants

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

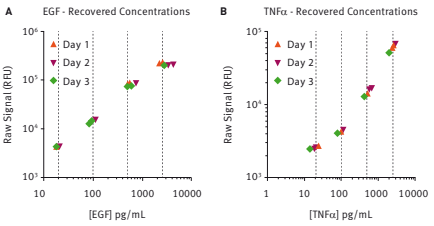

Fig. 3: Analyte recovery measurements for EGF (A) and TNFα (B).

Each day, either recombinant human EGF (Fig. 3A), recombinant human IL-2 (data not shown) or recombinant human TNFα (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 ELISA*ONE* assay plates (n=2 wells/concentration point). ELISA*ONE* antibody mix was added to the wells, and the assays were incubated for one hour, washed, and incubated with ELISA*ONE* 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.

Conclusion

This application note establishes ELISA*ONE*™ (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.