Labrox Application Note #003

Labrox® multimode plate reader:

DHEA ELISA assay performed on the Labrox multimode reader

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Introduction

The Enzyme-linked immunosorbent assay technique (ELISA) is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. The clinical impact of ELISA as nonradioactive variant of immunoassays is indeed overwhelming (1) In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, usually a polystyrene microtiter plate, and then an antibody with specificity for that antigen is applied over the surface so that it can bind to the antigen. The antibody is linked to an enzyme, and in the final step a substance that the enzyme can convert to some detectable signal (most commonly a colour change in a chemical substrate) is added. The intensity of this signal indicates the quantity of antigen in the sample (2).

Labrox® multimode plate readers (Fig. 1) are developed for several applications. Versatile and easy to use they include various detection modes, among them Absorbance (ABS). In this application note, we demonstrate that Labrox readers in ABS mode are appropriate to measure ELISA tests results with accuracy. In addition, we show that shaking and incubation steps of the protocol can also be performed using the plate reader shaking and temperature control features, with no need for further equipment.

Figure 1. Labrox multimode plate reader.

Materials

- ENZO DHEA Elisa Kit (cat. # ADI-900-093) 96 well kit
- Lonza AccuGENE™ Molecular Biology Water cat. BE51200 (to dilute the wash buffer)
- Micropipettes and tips; assay tubes
- Labrox multimode plate reader

Methods

The protocol provided with the kit was followed, with the only difference that 3 replicates of each standard concentration were measured instead of 2. The washes were manually performed, taking extreme care that plates were totally free of any residuals after the final wash.
Shaking was performed in an orbital shape mode, using the Labrox reader. Incubation at 37°C was also performed in the Labrox reader.

Standards preparation was done as instructed in the kit´s protocol (Table 1)

Table 1. Dilution Table for standards

Standard	Diluent Vol. (µl)	Vol. Added (µl)	DHEA conc. (pg/ml)
1	900	100, stock	50.000
2	750	250, Std. 1	12.500
3	750	250, Std. 2	3.125
4	750	250, Std. 3	781.25
5	750	250, Std. 4	195.31
6	750	250, Std. 5	48.83
7	750	250, Std. 6	12,21

Assays were performed according the kit´s assay protocol flow chart (Table 2)

Table 2. Assay protocol flow chart

	Blank	TA	NSB	Bo	Stds.
Well I.D	A1, B1,	C1,D1,F4	E1, F1,G4	G1,H1,H4	Stds. A2-E4
Std. Diluent	X	X	100 µl	100 µl	X
Assay buffer	X	X	50 µl	X	X
Std.	X	X	X	X	100 µl
conjugate	X	X	50 µl	50 µl	50 µl
antibody	X	X	X	50 µl	50 µl
Inc. 2 h RT shaking	→→	→→	→→	→→	→→
wash 3x	→→	→→	→→	→→	→→
1:10 dil. Conjugate	X	5 µl	X	X	X
substrate	200 µl	200 µl	200 µl	200 µl	200 µl
Inc. 3h@37 C, sealed	→→	→→	→→	→→	→→
stop solution	50 µl	50 µl	50 µl	50 µl	50 µl

Three replicates of each standard concentration were added to the plate. Blank, TA (total activity), NSB (nonspecific binding) and B_0 (0 pg/ml standard) wells were also prepared. Immediately after addition of the stop solution, the absorbance was measured at 405 nm and a full spectrum was run.

Results

Table 3. DHEA ELISA assay results

Sample	Average OD	Net OD (S/B)	SD	SD %	% Bound	DHEA conc.
Blank	**0,11**	**0,00**	**0,00**	**2**	0	
TA	**1,95**	**1,84**	**0,14**	**7**		
NSB	**0,11**	**0,00**	**0,01**	**5**	0	
Bo	**0,65**	**0,54**	**0,02**	**3**	100	
Std. 1	0,13	0,02	0,00	1	3,7	50000
Std. 2	0,17	0,06	0,00	0	11,1	12500
Std. 3	0,27	0,16	0,00	1	29,6	3125
Std. 4	0,38	0,27	0,02	4	50	781,25
Std.5	0,50	0,39	0,01	1	72,2	195,31
Std. 6	0,58	0,47	0,02	4	87	48,83
Std. 7	0,60	0,49	0,00	0	90,7	12,21

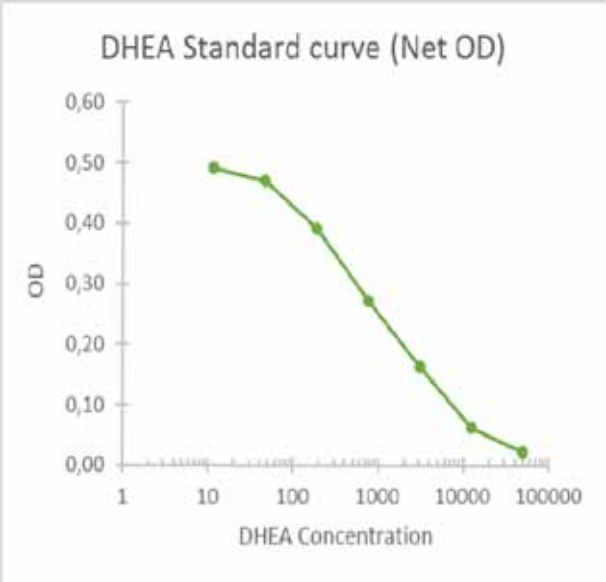

Figure 2. DHEA standard curve (Net OD)

Conclusions

The results show that the Labrox reader ABS mode can accurately read ELISA assays results. In DHEA ELISA the intensity of the yellow colour generated and read at 405 nm is expected to be inversely proportional to the DHEA concentration in standards and samples. That was in fact what we got, as shown in Table 3 and Fig. 2. The Standard curve follows perfectly the expected shape, with OD of approximately 0,5 for the higher DHEA concentration (50000 pg/ml) and 0,02 for the lowest concentration (12,21 pg/ml).

We also demonstrate that the shaking and temperature control functions of the Labrox reader can be utilized on the running of the protocol, with no need for any further equipment to run the assays.

References

1. Rudolf M. Lequin, “Enzyme Immunoassay (EIA)/ Enzyme-Linked Immunosorbent Assay (ELISA)”. Clinical Chemistry, December 2005, vol. 51 no. 12, 2415-2418.

2. ELISA Encyclopedia, Sino Biological Inc. http://www.clinchem.org/content/51/12/2415.full

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AN#003 Ver. 01
04. 2016