Fast and accurate detection of Alzheimer's Disease targets with SimpleStep ELISA® kits and SPECTROstar® *Nano*

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- **Assessing expression of neuronal targets is an important part in Alzheimer's disease research**
- **SimpleStep ELISA® Kits quantify BDNF, Tau and TREM2 in a one-wash 90 min protocol**
- **SPECTROstar® *Nano* detects assays and results are analyzed automatically with MARS analysis software**

Introduction

Alzheimer's disease (AD) is a multifactorial neurodegenerative condition. While it is accepted that AD results in progressive dementia, the causes and mechanisms leading to this condition are still poorly understood[1,2]. Numerous candidates for potential intervention have been proposed, but further research on their roles in AD and normal neural function is required to develop safe and effective therapies. Here, we describe the detection of 3 targets; Human BDNF (Brain derived neurotrophic factor), Tau and TREM2 (Triggering receptor expressed in myeloid cells 2) for which changes in expression have been implicated in AD progression. Each of the analytes was tested using the innovative SimpleStep ELISA® kits and detected with the SPECTROstar *Nano* microplate reader. SimpleStep ELISA® technology streamlines ELISA experiments to a semi-homogeneous format that results in a simple 90-minute, single-wash protocol (Figure 1). Combining these technologies with MARS data analysis allows you to get great results in the fastest, easiest way possible.

Assay Principle

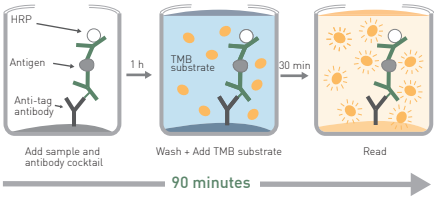

Fig. 1: SimpleStep ELISA® kits Assay Principle. The first step involves the addition of samples as well as a mixture of the capture and detector antibodies. A sandwich complex of these components is formed in solution, which binds to the immobilization anitbody coated on the plate via an affinity tag. Absorbance measurement is collected after a single wash step followed by color development.

Materials & Methods

- SPECTROstar® *Nano*
- SimpleStep ELISA® kits
  - Human BDNF ELISA Kit (ab212166)
  - Human Tau ELISA Kit (ab210972)
  - Human TREM2 ELISA Kit (ab224881)

Product Name	Human BDNF ELISA Kit	Human Tau ELISA Kit	Human TREM2 ELISA Kit
Catalog number	ab21266	ab210972	ab224881
Sensitivity (pg/ml)	2.4	3.3	10.5
Dynamic Range (pg/ml)	15.6-1000	31.25-2000	78.1-5000
Species Reactivity*	Hu, Ms, Rt	Hu, Rt, Bv	Hu
Sample type#	CS, S, HP, EP, CP	CS, S, CE, HP, EP, CP, CSF	S, CE, TE, CS, HP, EP, CP, CSF
Assay Time	1h 30 min	1h 30 min	1h 30 min
Intra-assay variability	2.80%	2.90%	6.30%
Inter-assay variability	5.30%	4.80%	4.90%

* CS: Cell Culture Supernatant; S: Serum; HP: Heparin Plasma; EP: EDTA Plasma; CP: Citrate Plasma; CE: Cell Culture Extracts; TE: Tissue Extracts; CSF: Cerebral Spinal Fluid. # Hu: Human; Ms: Mouse; Rt: Rat; Bv: Bovine

Experimental procedure

For all kits, samples were handled in accordance with instructions[3]. For all tests, standard curves were prepared based on an 8-point , 2-fold dilution series using duplicate samples. Starting concentrations were 5000, 2000 and 1000 pg/mL for TREM2, Tau and BDNF, respectively. Two different dilutions of biological replicates and blank replicates were used to determine assay reproducibility. The biological replicates were: human citrate plasma for BDNF, human brain extract for Tau and human serum for TREM2. Plates were read on a SPECTROstar *Nano* with the following settings:

Instrument Settings

Optic settings	Absorbance spectrum, Endpoint test	
	Wavelength range (step width)	400-700 (2) nm
General settings	Number of flashes	45
	Settling time	0.2 s

Data analysis

A MARS data analysis template was used to select appropriate wavelengths from spectral data set, generate a 4-parameter fit curve based on reference corrected data (OD_{450} – OD_{630}), assess %CV of blanks and recalculated

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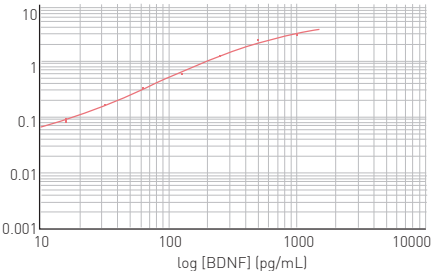
biological replicates, and determine MDD (minimum detectable dose) based on the formula: MDD = recalculated concentration of $Avg_{Blank}+2*SD_{Blank}$

Results & Discussion

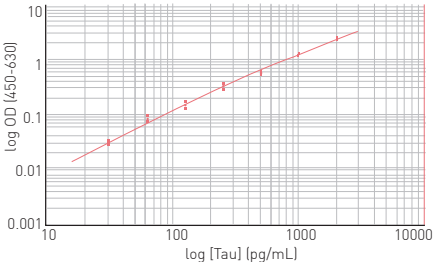
BDNF is a growth factor in the neurotrophin family. It is best characterized for promoting survival and maintenance of neurons, thus decreased BDNF might participate in the disrupted function of neurons associated with AD[2].



MDD	%CV of blanks (n=15)	%CV of bio-replicates	
		10% Plasma Citrate (n=8)	5% Plasma Citrate (n=8)
<7.86 pg/ml	3.7	5.2	2.7

Fig. 2: Fit curve, sensitivity and reproducibility from Human BDNF ELISA Kit (ab212166). The standard curve exhibits an R^2 of 0.997 for the 4-parameter fit.

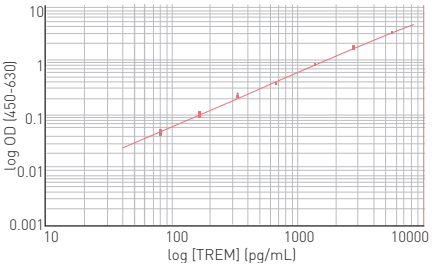
Tau proteins are associated with the microtubule network in neurons and as such are implicated in maintaining the unique structure of these cells which is vital for neuronal function. Tau is the protein component of neurofibrillary tangles, the most recognizable alterations associated with AD, which expression decreases with age[1].



MDD	%CV of blanks (n=15)	%CV of bio-replicates	
		2 ug/ml brain extract (n=8)	1 ug/ml brain extract (n=8)
19.4 pg/ml	5.2	4.4	6.4

Fig. 3: Fit curve, sensitivity and reproducibility from Human Tau SimpleStep ELISA® kit (ab210972). Standard curve exhibits great correlation to the 4-parameter fit (R^2 =0.997).

TREM2 is a receptor expressed exclusively by brain immune cells. Elevated levels of TREM2 is a biomarker for increased AD risk[1]. (Fig. 4).



MDD	%CV of blanks (n=15)	%CV of bio-replicates	
		2.5% Human Serum (n=8)	5% Human Serum (n=8)
39.6 pg/mL	10.1	2.2	1.1

Fig. 4: Fit curve, sensitivity and reproducibility from Human TREM2 ELISA Kit (ab224881). Standard curve exhibits excellent correlation to the 4-parameter fit (R^2 =0.9994)

Conclusion

SimpleStep ELISA® technology provides fast sample analysis with little hands-on time required. The SimpleStep ELISA® protocol only requires one washing step, providing results in just 90 minutes while delivering high sensitivity, specificity and reproducibility. Each single kit has been validated using biological samples to ensure it works the first time with high performance standards. The combination of SimpleStep ELISA® kits, SPECTROstar Nano microplate reader and MARS data analysis templates provide further benefits in performance, saving significant time and effort. These tools offer immediate and accurate results to support current and future neuroscience research needs.

References

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2. Fumagalli, F., Racagni, G., Riva, M.A. The expanding role of BDNF: a therapeutic target for Alzheimer's disease? Pharmacogenomics J. (2006) **6**: 8-15
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