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CD Microplate Reader Application Note

- Selected examples for CD measurements

I – INTRODUCTION

Circular dichroism (CD), the commonly used technique for chiral analysis, refers to the differential absorption between left and right circularly polarized light. It is often used for assigning the secondary structures of proteins and determining enantiomeric purities in asymmetric syntheses, both of which require the ability to do the measurements in a high-throughput fashion as needed by the Anslyn and Kahr groups[1,2].

Traditional CD spectrometers use a horizontal light path which require transferring sample into a cuvette for measurement from a traditional well plate where the asymmetric syntheses were performed, or the proteins modified. Even though this process is expedited when robotic liquid handling systems are coupled to a conventional CD spectrometer, the cuvette must be cleaned between measurements. This is still a laborious and time-consuming process.

The CD Microplate Reader is a vertical CD spectrometer that turns the measuring light beam from the horizontal direction to vertical allowing for the use of a computer-controlled XY stage so that CD signals are read from a well plate directly.

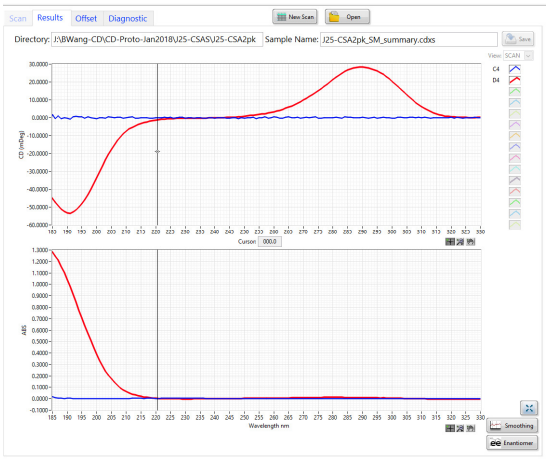
The CD Microplate Reader thus eliminates the time-consuming processes of 1) transferring the contents from each well of a well plate into a cuvette, and 2) cleaning the cuvette between measurements, significantly increasing productivity, as much as 100-fold with respect to conventional CD systems coupled to a robot[1,2,3].

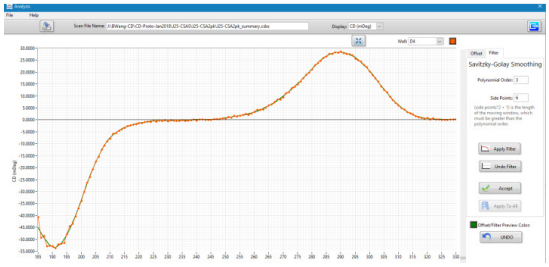
Several types of commercial well plates are available for use with the CD Microplate Reader. Well plates with a glass bottom can be used in the visible spectral region. Well plates with a fused silica bottom can be used in the ultraviolet and visible spectral regions. Well plates made from solid fused silica provide the best durability and performance.

In this paper, we provide several measurement examples using the CD Microplate Reader.

II – SELECTED EXAMPLES FOR CD MEASUREMENTS IN MICRO-WELL PLATE

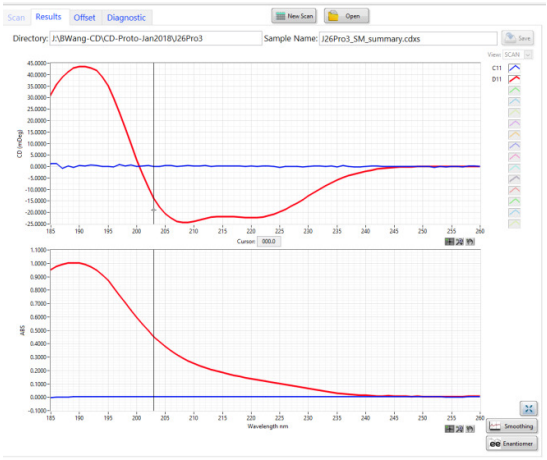
Fig. 1. D-(+)-10-camphorsulfonic acid (CSA)

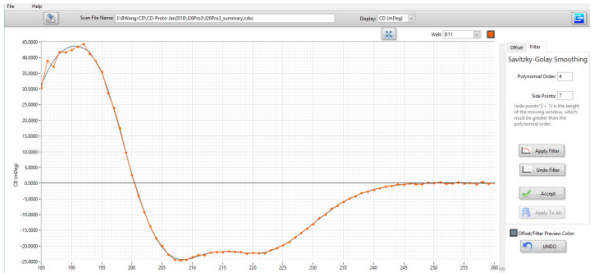




D-(+)-10-camphorsulfonic acid (CSA)	
Sample	(+)-CSA
Solvent	Deionized water
Concentration	0.2 mg/ml
Well-plate used	Hellma solid quartz 96 well (White)
Volume in well	200 uL
Wavelength	185nm – 330nm
Time integration per point	3 s
Step size	1 nm
Nitrogen flow rate	0.5 L/min

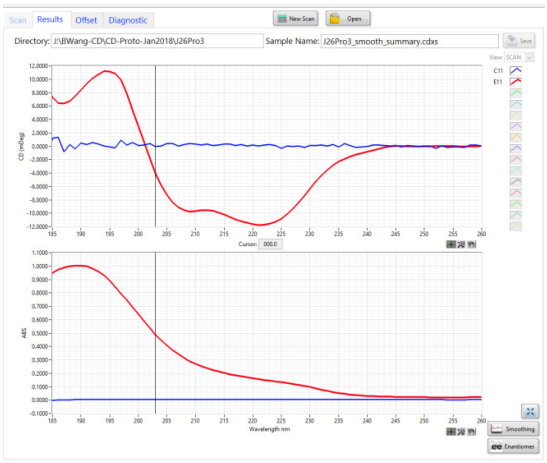
Fig. 2. Bovine serum albumin (BSA)

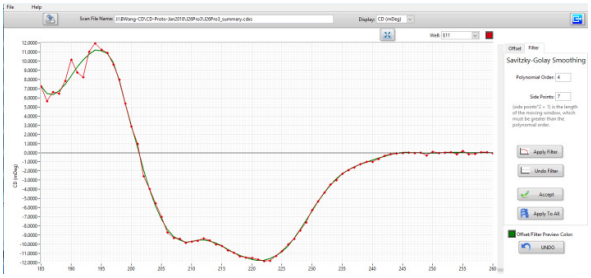




Bovine serum albumin (BSA)	
Sample	Bovine serum albumin (BSA)
Solvent	Deionized water
Concentration	0.05 mg/ml
Well-plate used	Hellma solid quartz 96 well (White)
Volume in well	80 uL
Wavelength	185nm – 260nm
Time integration per point	3 s
Step size	1 nm
Nitrogen flow rate	0.5 L/min

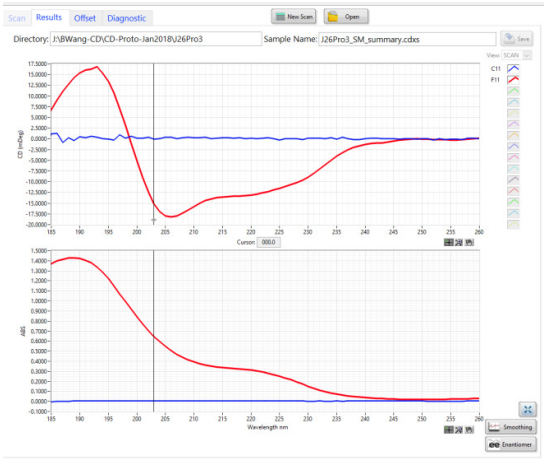
Fig. 3. Cytochrome C

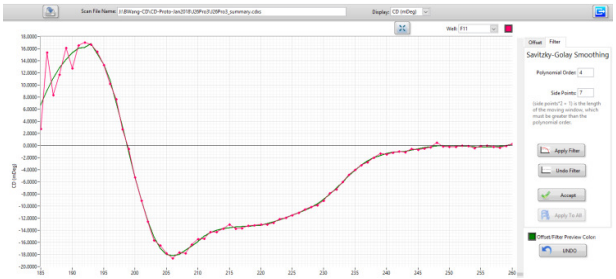




Cytochrome C	
Sample	Cytochrome C
Solvent	Deionized water
Concentration	0.1 mg/ml
Well-plate used	Hellma solid quartz 96 well (White)
Volume in well	80 uL
Wavelength	185nm – 260nm
Time integration per point	3 s
Step size	1 nm
Nitrogen flow rate	0.5 L/min

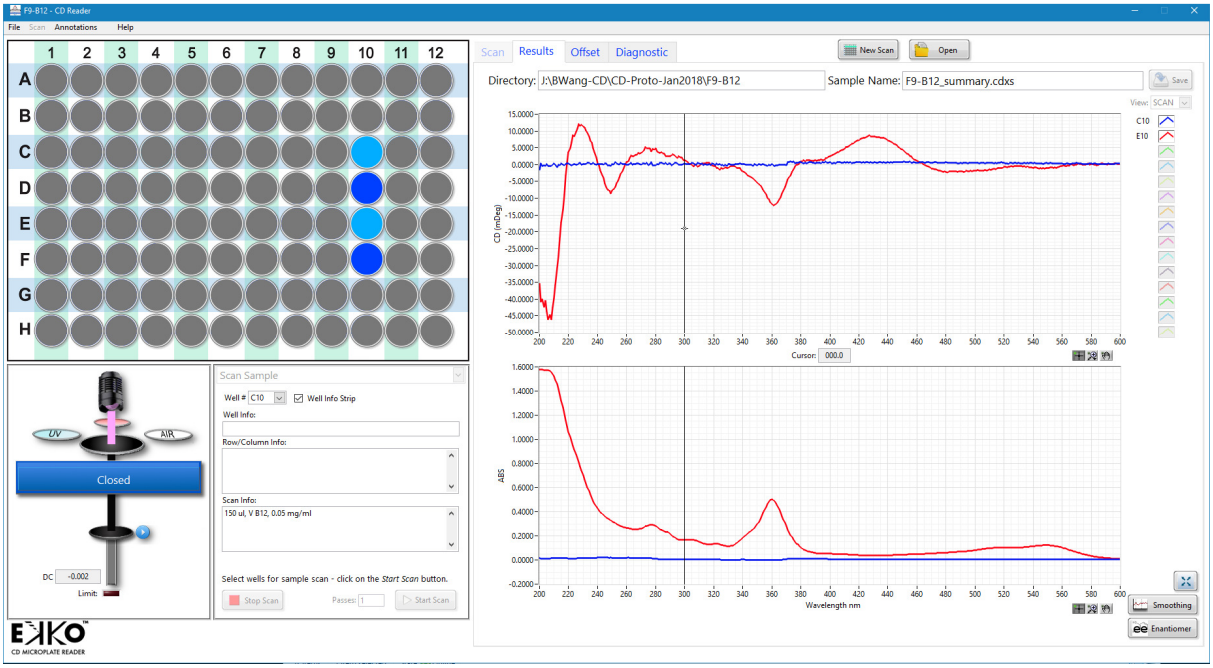
Fig. 4. Lysozyme from egg white





Lysozyme from egg white	
Sample	Lysozyme from egg white
Solvent	Deionized water
Concentration	0.1 mg/ml
Well-plate used	Hellma solid quartz 96 well (White)
Volume in well	80 uL
Wavelength	185nm – 260nm
Time integration per point	3 s
Step size	1 nm
Nitrogen flow rate	0.5 L/min

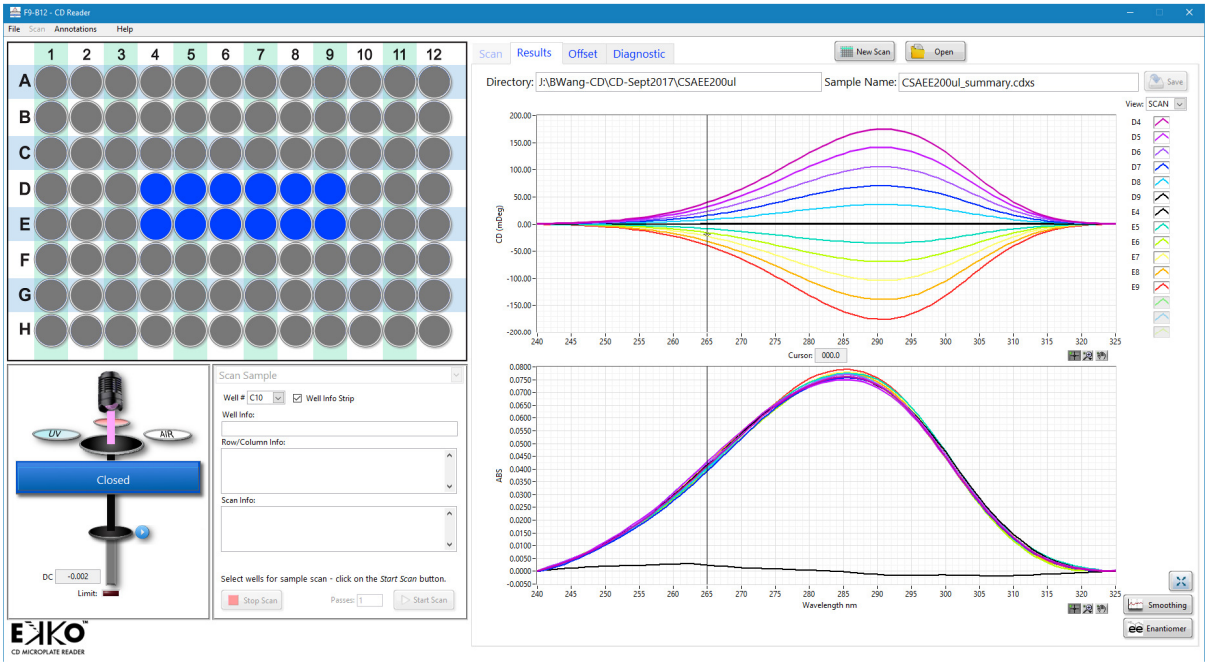
Fig. 5. Vitamin B_{12}

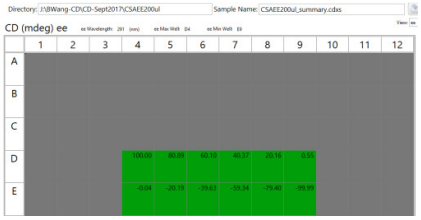


C10	150 ul water	E10	150 uL Vitamin B_{12} solution

Vitamin B_{12}	
Sample	Vitamin B_{12}
Solvent	Deionized water
Concentration	0.05 mg/ml
Well-plate used	Hellma solid quartz 96 well (White)
Volume in well	150 uL
Wavelength	200nm – 600nm
Time integration per point	3 s
Step size	1 nm
Nitrogen flow rate	0.5 L/min

Fig. 6. Enantiomeric excess measurement for (+) and (-) - camphorsulfonic acid (CSA)

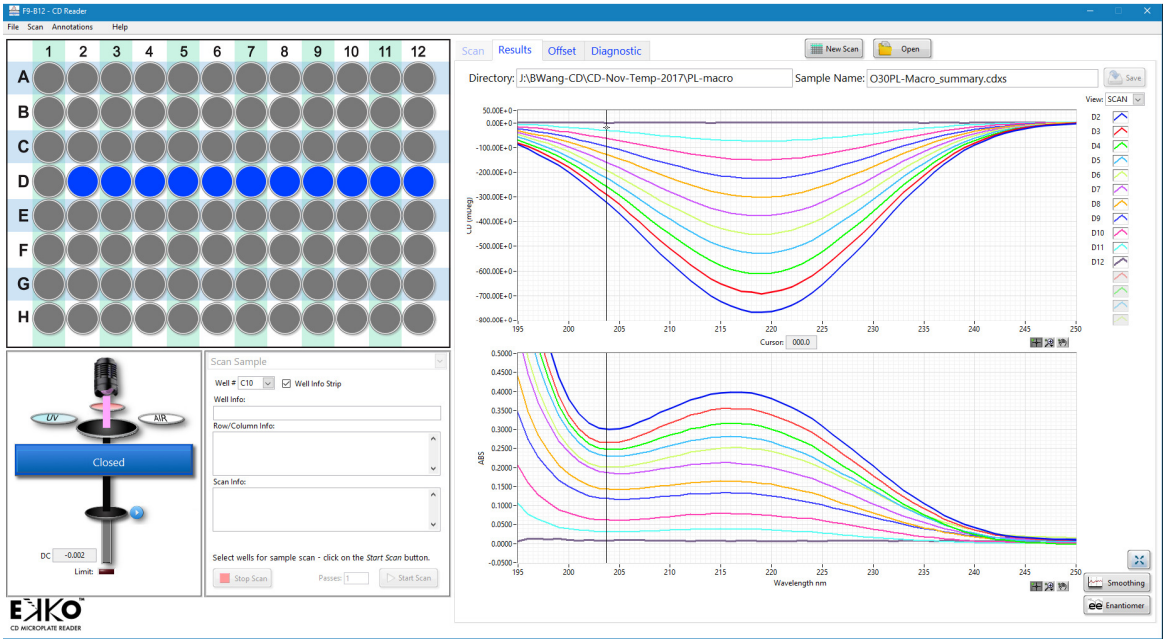




CSA (+)uL/CSA(-)uL	4	5	6	7	8	9
D	200/0	180/20	160/40	140/60	120/80	100/100
E	water	80/120	60/140	40/160	20/180	0/200

CSA enantiomeric excess	
Sample	(+)-CSA and (-)-CSA
Solvent	Deionized water
Concentration	~1 mg/ml
Well-plate used	Hellma solid quartz 96 well (White)
Volume in well	200 uL
Wavelength	240nm – 325nm
Time integration per point	3 s
Step size	1 nm
Nitrogen flow rate	0.5 L/min

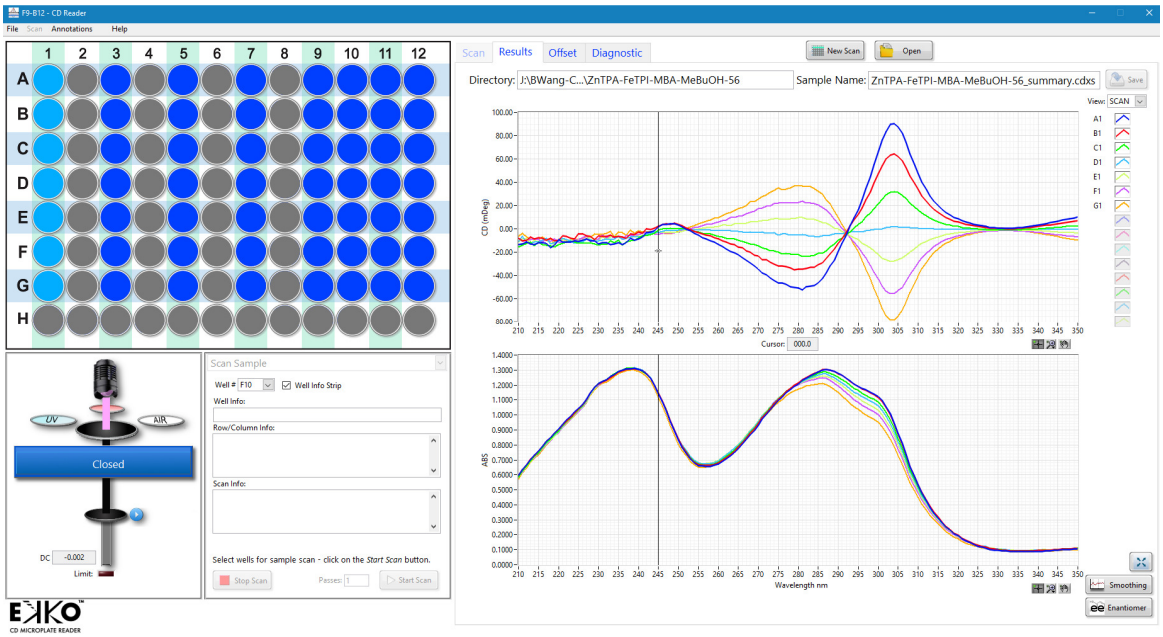
Fig. 7. D-(-)-pantolactone



Well layout	2	3	4	5	6	7	8	9	10	11	12
D(H_2O)	200	180	160	140	120	100	80	60	40	20	0
D(PL)	0	20	40	60	80	100	120	140	160	180	200
PL: D-(-)-pantolactone											

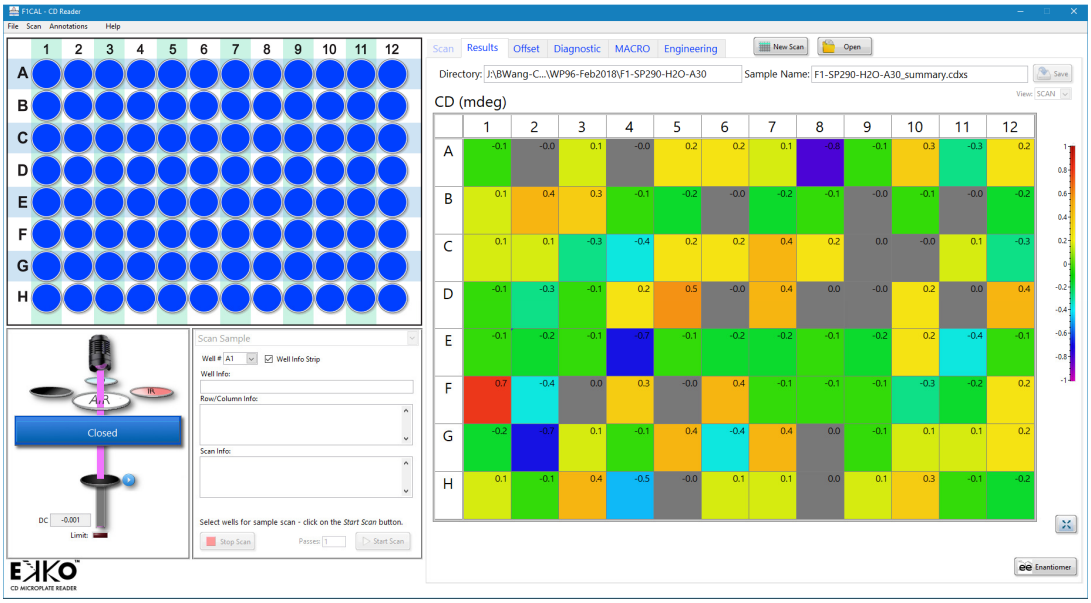
D-(-)-pantolactone at different concentrations	
Sample	D-(-)-pantolactone
Solvent	Deionized water
Concentration	1 mg/ml in well D2
Well-plate used	Hellma solid quartz 96 well (White)
Volume in well	200 uL
Wavelength	195nm – 250nm
Time integration per point	3 s
Step size	1 nm
Nitrogen flow rate	0.5 L/min

Fig. 8. Amine assembly formed with 3-methylpyridine-2-carbaldehyde, methylbenzylamine, and Fe(II).



Data taken by Prof. Anslyn's group at the University of Texas at Austin using an earlier version of the CD Reader.

Fig. 9. Water blank CD measured at 290 nm for all 96 wells (3s/well)



Mean: -0.0089 mdeg	Standard deviation: 0.26 mdeg

III – SUMMARY

1. The CD Microplate Reader can be used to measure small chiral molecules as well as large biological molecules such as proteins.

2. The CD Microplate Reader is ideal for studying combinatory mixing of reagents, catalysts, solvents, and various experimental conditions in micro-well plates.
3. At a desired wavelength, CD Microplate Reader can measure CD values from all 96 wells in less than 2 minutes (without averaging). The user can easily select an integration time for lower noise measurements.

REFERENCES

1) Metola, P., Nichols, S.M. Kahr, B., and Anslyn, E.V., Well plate circular dichroism reader for the determination of enantiomeric excess. *Chem. Science.* 5, 4278-4282 (2014).
2) Jo, H.H., Cao, X. You, L., Anslyn, E.V., and Krische, M.J., Application of high-throughput enantiomeric excess optical assay involving a dynamic covalent assembly: parallel asymmetric allylation and ee sensing of homoallylic alcohols. *Chem. Science.* 6, 6747-6753 (2015).
3) Fielder, S., Cole, L., and Keller, S., Automated Circular Dichroism spectroscopy for medium throughput analysis of protein conformation. *Anal. Chem.* 85, 1868-1872 (2013).
4) Hussain, R., Javorfi, T., Rudd, T. R., and Siligardi, G., High-throughput SRCD using multi-well plates and its applications. *Nature, Sci Reports* 6, 38028 (2016).