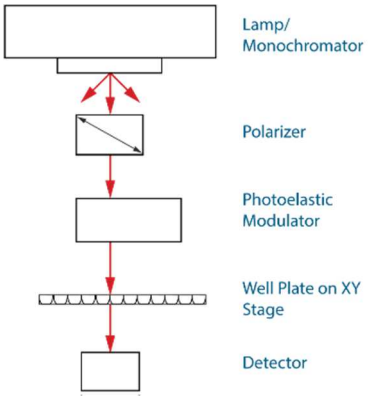
# EKKO™ CD Microplate Reader

## Allowed Sample Types - Selected examples for CD measurements

## I – INTRODUCTION

Circular dichroism (CD) is commonly used to assign the secondary structures of proteins and determining enantiomeric purities in asymmetric syntheses, both of which benefit from the ability to do the measurements in a high-throughput fashion [1,2]. CD relies on the differential absorption between left and right circularly polarized light about a chiral center.

The primary advantage of the EKKO™ CD Microplate Reader is its speed resulting from the use of well plates allowing for the highest throughput possible. It accomplishes this by turning the light path from the horizontal used in traditional CD systems to vertical, allowing for a computer controlled XY stage so that CD signals are read directly from a well plate instead of from a cuvette.




This removes the time-consuming steps of 1) transferring the contents from each well of a well plate into a cuvette, and 2) cleaning the cuvette between measurements. As a result, it takes only two minutes to measure the CD signal in all 96 wells of a standard well plate at any given single wavelength and needs less than 90 minutes to measure all 96 CD spectra over 50 wavelengths in a standard well plate. This results in a significant increase in productivity, as much as 100-fold with respect to conventional CD systems coupled to liquid handling robotics[1,2,3].

However, unlike conventional CD systems, given that the geometry of only one side of the light path is dictated by glass, it is susceptible to concerns which are not present for standard technologies including: meniscus effects, variable length light paths throughout the course of the measurement due to evaporation or misloading of the well and variations in the optical features of the glass or silica. Rightly so, given the above considerations, there are uncertainties about the type of molecules which can be measured using the EKKO™ CD Microplate Reader.

In this technical note, we demonstrate measurements for several CD standards covering the various classes of molecules that circular dichroism is used to study. Enantiomeric excess measurements for CSA and the unique in plate assays not available for traditional CD systems are also demonstrated.

## II – Select Examples of CD Measurements

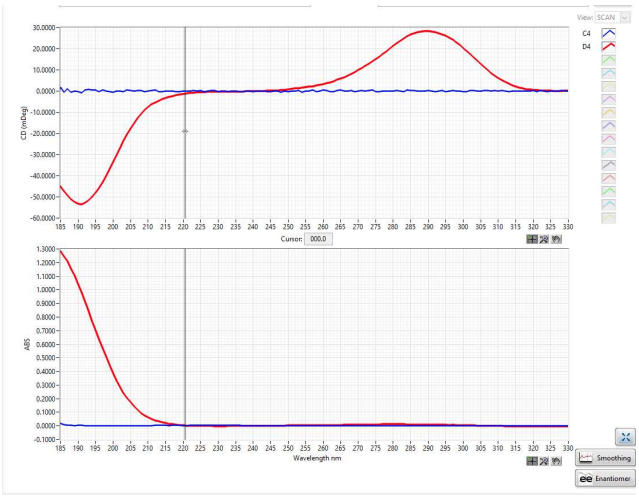


**Fig. 1. Raw CD (top) and Abs (bottom) spectra of CSA.** 200 µl of CSA (Sigma) at 0.2 mg/ml in well #D4 of a solid fused silica 96 well plate (Hellma). Raw spectra were collected from 185 – 330 nm in 1 nm steps with an integration time of 3 seconds at a room temperature of 25°C.

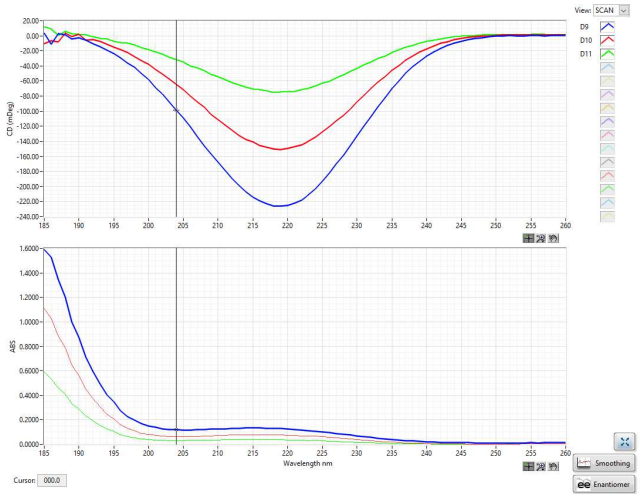


**Fig. 2. Raw CD (top) and Abs (bottom) spectra of a concentration series of PL.** 200 µl of 300 µg/ml, 200 µg/ml, 100 µg/ml PL(Sigma) in wells #D9-D11 of a solid fused silica 96 well plate (Hellma). Raw spectra were collected from 195 – 250 nm in 1 nm steps with an integration time of 3 seconds at a room temperature of 25°C.

Figures 1 & 2 demonstrate the capabilities of the EKKO™ CD Microplate Reader for smaller chiral molecules.

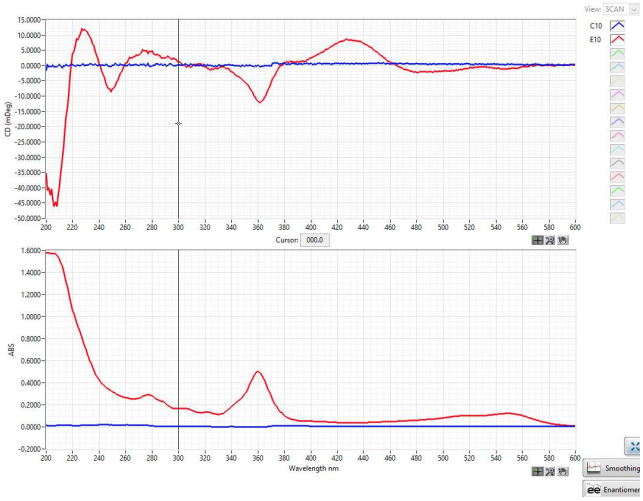


**Fig. 3. Raw CD (top) and Abs (bottom) spectra of Vitamin $B_{12}$.** 150 µl of B12 (Sigma) at 0.1 mg/ml in well #D10 of a solid fused silica 96 well plate (Hellma). Raw spectra were collected from 200 – 600 nm in 1 nm steps with an integration time of 3 seconds at a room temperature of 25°C.

Figure 3 demonstrates the capabilities of the EKKO™ CD Microplate Reader for larger complicated chiral molecules.

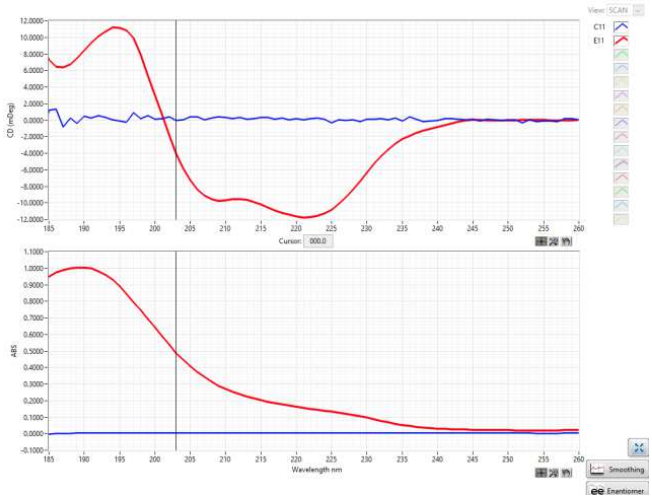


**Fig. 4. Smoothed CD (top) and Abs (bottom) spectra of Cytochrome C.** 80 µl of Bovine Heart Cytochrome C (Sigma) at 0.1 mg/ml in well #E11 of a solid fused silica 96 well plate (Hellma). Raw spectra were collected from 185 – 260 nm in 1 nm steps with an integration time of 3 seconds at a room temperature of 25°C and smoothed with 3 point 3rd order Savitzky-Golay function.

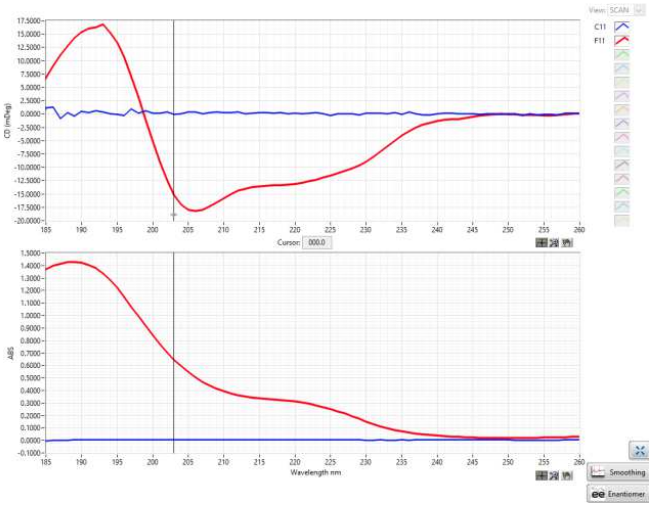


**Fig. 5. Smoothed CD (top) and Abs (bottom) spectra of Lysozyme.** 80 µl of Hen Egg White Lysozyme (Sigma) at 0.1 mg/ml in well #F11 of a solid fused silica 96 well plate (Hellma). Raw spectra were collected from 185 – 260 nm in 1 nm steps with an integration time of 3 seconds at a room temperature of 25°C and smoothed with 3 point 3rd order Savitzky-Golay function.

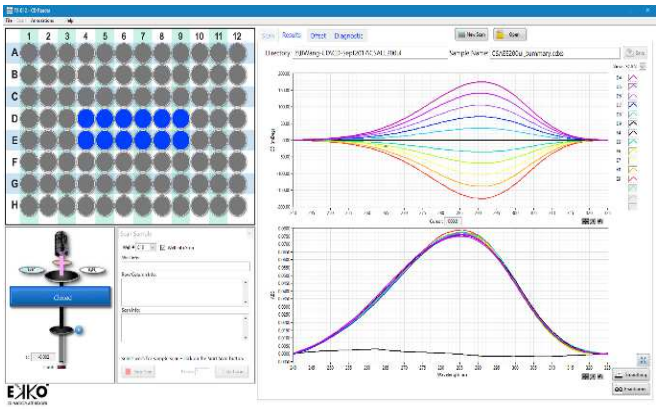


**Fig. 6. Enantiomeric excess measurements for CSA.** 200 µl of of various combinations of (+) & (-) CSA (Sigma) at total concentration of 0.1 mg/ml in wells #D4-E9 of a solid fused silica 96 well plate (Hellma). Raw spectra were collected from 240 – 325 nm in 1 nm steps with an integration time of 3 seconds at a room temperature of 25°C.

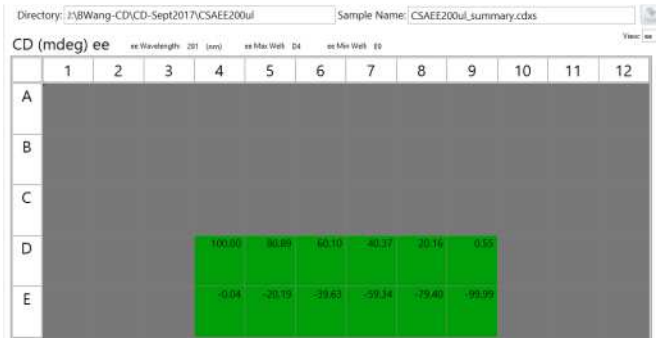



**Fig. 7. Enantiomeric excess measurement analysis for CSA.** 200 µl of various combinations of (+) & (-) CSA (Sigma) at a total concentration of 0.1 mg/ml in wells #D4-E9 of a solid fused silica 96 well plate (Hellma). Raw spectra were collected from 240 – 325 nm in 1 nm steps with an integration time of 3 seconds at a room temperature of 25°C. EE Excess analysis was performed using the built-in function in the CD Reader Software.

Figures 6 & 7 illustrate the built-in functionality for determining Enantiomer Excess applications of the EKKO™ CD Microplate Reader.

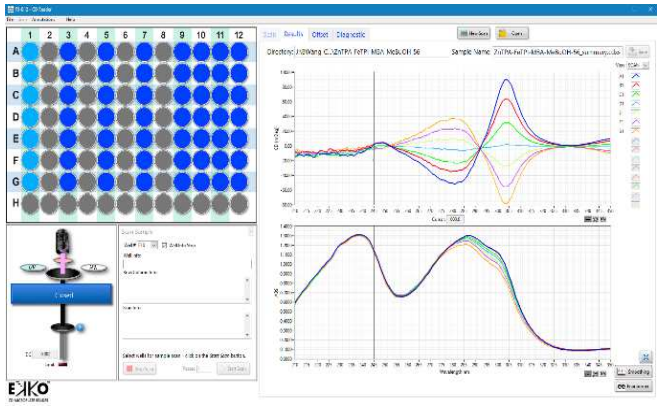


**Fig. 7. Asymmetric Synthesis Determinations in reaction vessel.** Amine assembly formed with 3-methylpyridine-2-carbaldehyde, methylbenzylamine, and Fe(II) in a solid silica bottom 96 well plate with black sidewalls (Porvair) courtesy of Eric Ansyln.

Figure 7 demonstrates the unique capabilities of the EKKO™ CD Microplate Reader for measurements of asymmetric chiral synthesis in the reaction well plate.

## III – SUMMARY & RECOMMENDATIONS

1. The EKKO™ CD Microplate Reader can be used to measure small chiral molecules as well as large biological molecules such as proteins.
2. The EKKO™ 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, EKKO™ CD Microplate Reader can measure CD values from all 96 wells in less than 2 minutes (without averaging).
4. Across a spectrum with 50 desired wavelengths, the EKKO™ CD Microplate Reader can measure CD values from all 96 wells in less than 90 minutes (without averaging).

## 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).