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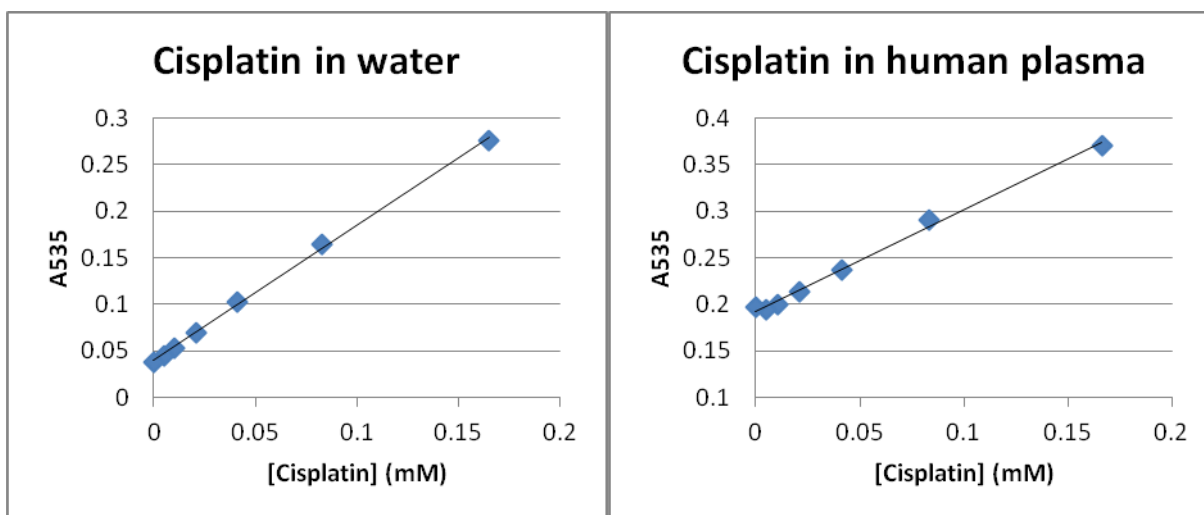
INSTRUCTIONS

ProFoldin MicroMolar Cisplatin Assay Kit

CATALOG NUMBER CPT200

INTRODUCTION

Cisplatin [or cis-diamminedichloroplatinum, $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$] is the first member of platinum-containing anti-cancer drugs. It binds DNA and causes DNA crosslinking which ultimately triggers apoptosis (programmed cell death). The MicroMolar Cisplatin Assay Kit (Catalog number CPT200) is designed for high throughput measurement of micromolar concentrations of Cisplatin. The assay is based on the light absorbance at 535 nm. The assay kit can be used for assays of cisplatin in drug discovery, drug development, pharmaceutical samples and biological samples.



The MicroMolar Cisplatin Assay Kit (Catalog number CPT200) includes 4 ml of Reagent A, 400 μl of 10 x Reagent B, and 2 ml of 10 x Reagent C. It is for 200 assays using 96-well plates. Cuvettes may also be used for measurements.

ASSAY PROTOCOL

The following assay protocol is based on using a 96-well plate for the measurement. The sample volume is 100 μl and the final assay volume is 240 μl . The assay reactions and measurement are on a transparent 96-well plate. It is a high throughput assay format.

For assays using cuvette, the sample volume is 400 μl and the final assay volume is 960 μl . The assay reactions are carried out in eppendorf tubes or test tubes. The samples are transferred into a cuvette for measurement of the light absorbance.



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STANDARD CURVE

1. **Sample preparation:** Prepare 100 μ l of cisplatin solutions in the wells of a transparent 96-well plate with a two-fold serial dilution from 0.1 mg/ml to zero in water. For each 10 assays, dilute 20 μ l of 10 x Reagent B with 180 μ l of water to make 1 x Reagent B; dilute 100 μ l of the 10 x Reagent C with 0.9 ml of water to make 1 x Reagent C.

Note: If the 10 x Reagent B solution forms crystals during storage in a freezer, warm the solution with hands or water bath until the crystal is completely dissolved before preparing the 1 x Reagent B solution.

2. **Assay:** Into the wells with 100 μ l of cisplatin solutions, add 20 μ l of Reagent A and 20 μ l of 1 x Reagent B. Incubate the mixtures at 65°C for 30 min. Add 100 μ l of 1 x Reagent C into each well. Read the light absorbance at 535 nm (A_{535})

3. **Data Analysis:** Plot the A_{535} values and the Cisplatin concentration [**Cisplatin**] to generate the linear standard curve.

$$A_{535} = a [\text{Cisplatin}] + b$$

Where the A_{535} values are from experimental data, the **a** and **b** values are from the linear fitting between the A_{535} values and the Cisplatin concentrations.

UNKNOWN SAMPLES

Follow the same procedure to measure the light absorbance A_{535} values from the unknown samples. Calculate the Cisplatin concentrations in the unknown samples using the A_{535} values from the unknown samples and the **a** and **b** values from the standard curve.

$$[\text{Cisplatin}] = (A_{535} - b) / a$$

RELATED PRODUCTS

OPT200	MicroMolar Oxaliplatin Assay Kit
MPX200	MicroGram Polymyxin Assay Kit
CIP100	MicroGram Ciprofloxacin Assay Kit
CFZ200	MicroGram Carfilzomib Assay Kit
VAN100	MicroGram Vancomycin Assay Kit
PST100	Penicillin Drug Stability Test Kit

For more concentration assays of various biochemical molecules and inorganic ions, please visit our website at www.profoldin.com.
