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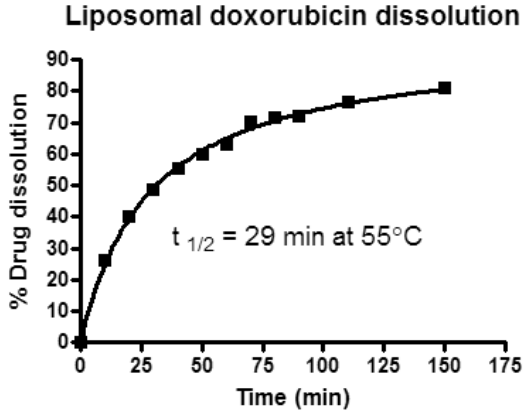
INSTRUCTIONS

ProFoldin
Liposome Drug Dissolution Assay Kit

CATALOG NUMBER **LDD05**

INTRODUCTION

Liposome drug dissolution is release of the encapsulated drug into the medium of the liposome. The in vitro liposomal drug dissolution test is performed under certain chemical and physical pressure to simulate the release of the encapsulated drug. For liposomes with encapsulated drugs by pH gradient remote loading, ammonium salt is added in the medium to accelerate the drug release. The **Liposome Drug Dissolution Assay kit (Catalog number LDD05)** is designed to analyze liposome drug dissolution under optimized conditions. The released drug is removed by spin-column and the intact liposome is quantified.



The **Liposome Drug Dissolution Assay kit (Catalog number LDD05)** includes 40 prepacked spin columns, 1.5 ml of 10 x dissolution buffer composed of 2 M $(NH_4)_2SO4$, pH 5.5 and 10 ml of elution buffer composed of 10 mM Histidine, pH 6.5, 9.2 % sucrose. It is for 5 drug dissolution assay tests of liposomal drug samples at the selected temperature.

ASSAY PROTOCOL

The following protocol is for dissolution test of liposomal doxorubicin containing 2 mg/ml doxorubicin in liposomes composed of 9.6 mg/ml hydrogenated soy phosphocholine lipid, 3.2 mg/ml cholesterol and 3.2 mg/ml DSPE-PEG2000. The drug dissolution condition depends on the composition of the liposome, dissolution buffer and temperature. Different dissolution buffers and temperature ranges should be tested for different liposomes. Typically, a condition under which approximately 50 % drug is released in 30 min is appropriate for the time-cause drug dissolution experiment.

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1. Sample preparation

(1) Remove any high aggregates by filtration of the samples through a 0.22 µm filter.
(2) Mix 1.6 ml of water, 0.2 ml of 10 x Dissolution buffer and 0.2 ml of 2 mg/ml liposomal doxorubicin. Divide the mixture in 8 aliquots, 0.24 ml each. Save one aliquot as the time-zero sample and keep it on ice in the dark.
(3) Incubate the other aliquots in 55°C water bath.
(4) Take one aliquot out of the water bath and save the heated samples on ice in the dark at time points (t = 15, 30, 45, 60, 90, 120, and 150 min).

2. Column preparation

(1) Briefly spin 8 columns using a benchtop Eppendorf centrifuge to set down the resin. Remove the caps of 1.5-ml Eppendorf tubes and use them as receiving tubes. Remove the bottom tip and the cap of each spin column and insert the column into a receiving tube.
(2) Spin the columns at 1000 rpm for 1 min and discard the elute.
(3) Repeat Step (2).
(4) Add 150 µl of the elution buffer, spin the columns at 1000 rpm for 2 min and discard the elute.
(5) Spin the columns at 1000 rpm for 4 min and change to clean receiving tubes.

3. Separation of the released drug

(1) Load 150 µl of each sample onto a column (one time-zero sample, seven heated samples)
(2) Spin the columns at 1000 rpm for 2 min.
(3) Add 50 µl of the elution buffer on the top of the column and spin the column at 1000 rpm for 4 min.
(4) Mix the elute well by pipetting up-and-down a few times.

4. Drug concentration measurement

(1) Use 150 µl of the elute and a 96-well plate to read the light absorbance of doxorubicin at 490 nm. Also use the elution buffer to measure the background.
(2) Subtract the background value from the reading values to get the net absorbance values.

5. Data processing for drug dissolution

(1) Calculate the drug percentage dissolution values: $D_t = 100\ \% - 100\ \% \times A_t / A_0$ where A_t is the net light absorbance of the heated sample at time pint t; A_0 is the net light absorbance of the zero-time sample.
(2) Plot the Drug dissolution (%) values D_t versus the time t and calculate the half life ($t_{1/2}$) using curve fitting using the fitting curve of $D_t = D_{max} \times t / (t_{1/2} + t)$.

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