



## ProFoldin

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# INSTRUCTIONS

## ProFoldin

# Lipid Bilayer Permeability Disruption Assay Kit

CATALOG NUMBER

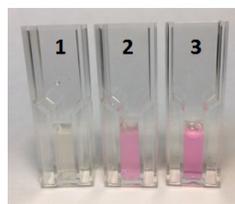
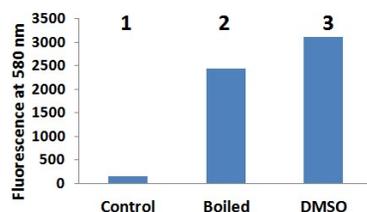
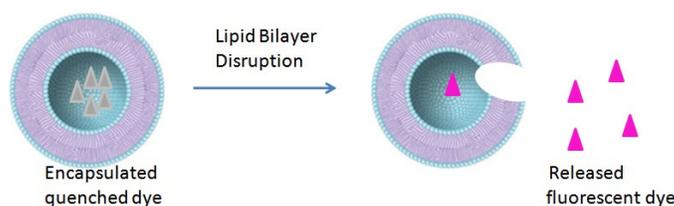
BPD100K

## INTRODUCTION

Disruption of cell membranes that are composed of lipid bilayers leads to dramatic biological effects. For example, pore-forming cytotoxins disrupt the cell membrane permeability causing the release of inflammatory cytokines and tissue damage. The Lipid Bilayer Permeability Disruption Assay employs a liposome-encapsulated dye to monitor the release of the dye due to damage of the integrity of the lipid bilayer. Since liposomes have the bilayer structure similar to cell membranes, this dye-releasing assay may be used to test compounds that disrupt the permeability and damage cell membranes. The released dye is monitored by fluorescence at 580 nm (excitation at 540 nm). It is a high throughput assay using 96-well plates and is suitable for screening of compound collections.

The liposome bilayer is composed of lipids HSPC: cholesterol: DSPE-PEG2000 (3:1:1 mass ratio) which is the same as the lipids for an existing liposomal drug in clinic. The dye is rhodamine conjugated with diethylenetriamine and its fluorescence is quenched inside of the liposome. When the bilayer permeability is disrupted, the encapsulated dye is released and the fluorescence is generated in the presence of the color development agent outside of the liposome.

### Lipid Bilayer Permeability Disruption Assay



The **Lipid Bilayer Permeability Disruption Assay Kit** (Catalog number BPD100K) includes 1.25 ml of liposomal dye, 1.25 ml of color development solution, 7.5 ml of buffer and 1 ml of DMSO. It is for assays of 100 samples using a 96-well plate.



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# INSTRUCTIONS

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## PROTOCOL

The following protocol is based on using a 96-well plate fluorescence plate reader (emission 580 nm, excitation 540 nm). The total sample volume is 100  $\mu$ l. Cuvettes may also be used for fluorescence measurement if the sample volume is adjusted accordingly.

### 1. Temperature effect on bilayer permeability

Heating disrupts bilayer permeability and releases the liposome-encapsulated dye. To test the temperature effect, in each test tube, add 75  $\mu$ l of Buffer, 12.5  $\mu$ l of liposomal dye and 12.5  $\mu$ l of color development solution. Incubate the tubes at different temperatures (e. g. boiling water bath, room temperature, etc) for 5 min. Transfer the samples into wells of a black 96-well plate and read the fluorescence at 580 nm (Excitation at 540 nm) in 10 min.

### 2. Control experiment of bilayer permeability disruption by DMSO

DMSO completely disrupts bilayer permeability and releases the liposome-encapsulated dye. In a well of a black 96-well plate, add 75  $\mu$ l of DMSO, 12.5  $\mu$ l of liposomal dye and 12.5  $\mu$ l of color development solution. Incubate the sample at room temperature for 15 min and read the fluorescence at 580 nm (Excitation at 540 nm).

### 3. Disruption of bilayer permeability by compounds

Some compounds may also disrupt bilayer permeability and release the liposome-encapsulated dye. In a well of a black 96-well plate, add the compound solution and Buffer to make a volume of 75  $\mu$ l then add 12.5  $\mu$ l of liposomal dye. Incubate the tube at a proper temperature for a selected time period. Finally add 12.5  $\mu$ l of color development solution and incubate the sample for 10 min. Read the fluorescence at 580 nm (Excitation at 540 nm).

## RELATED PRODUCTS

### Liposome products:

LDE10	Liposome Drug Encapsulation Assay Kit
LDD05	Liposome Drug Dissolution Assay Kit
LIP1000	MicroGram Lipid Assay Kit
SPS20	Liposome Plasma Stability Test Kit
PHPC200AS	Ready-to-load PEGylated HSPC Liposomes with Ammonium Sulfate
DPC100AS	Ready-to-load DPPC Liposomes with Ammonium Sulfate
DPC001FL	Liposomal Fluorescein Dye
DPC001AO	Liposomal Acridine Orange Dye
PHC001AO	PEGylated Liposomal Acridine Orange Dye
DPC001RG	Liposomal Rhodamine G Dye

### Nanodisc products:

SMA31-100MG	Styrene - Maleic Acid Copolymer 3:1 Free Acid- 100 mg
SMA21-100MG	Styrene - Maleic Acid Copolymer 2:1 Free Acid- 100 mg

For more information of liposome and nanodisc products, please visit our website at [www.profoldin.com](http://www.profoldin.com).