For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

# Mes-Red磷脂酶D检测试剂盒
# Mes-Red Phospholipase D Assay Kit

Do not eat Store at -20° C & in the dark.

**Cat.No.** MPD3965 **Size :** 500 tests

**Technical literature is available at :** www.mesgenbio.com.
**E-mail MesGen Technical Services if you have questions on use of this system :** tech@mesgenbio.com

## Introduction

The Mes-Red Phospholipase D Assay Kit provides a sensitive method for measuring phospholipase D (PLD) activity *in vitro* using a fluorescence microplate reader or fluorometer. In this enzyme-coupled assay, PLD activity is monitored indirectly using Mes-Red reagent, a sensitive fluorogenic probe for $H_2O_2$.First, PLD cleaves the phosphatidylcholine (lecithin) substrate to yield choline and phosphatidic acid. Second, choline is oxidized by choline oxidase to betaine and $H_2O_2$. Finally, $H_2O_2$, in the presence of horseradish peroxidase, reacts with Mes-Red reagent in a 1:1 stoichiometry to generate the highly fluorescent product,resorufin. Because resorufin has absorption and fluorescence emission maxima of approximately 571 nm and 585 nm, respectively, there is little interference from autofluorescence in most biological samples. The kit can be used to continuously assay PLD enzymes with near-neutral pH optima, whereas PLD enzymes with acidic pH optima can be assayed in a simple twostep procedure. Experiments with purified PLD from *Streptomyces chromofuscus* indicate that the Mes-Red PLD Assay Kit can detect PLD levels as low as 10 mU/mL using a reaction time of one hour. The kit is potentially useful for detecting PLD activity in cell extracts or for screening PLD inhibitors.

## Contents

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|---|---|
| Mes-Red reagent | 500 µL |
| 1X Reaction Buffer | 60 mL |
| HRP stock solution | 1 mL |
| Choline oxidase stock solution | 600 µL |
| $H_2O_2$ solution | 500 µL |
| Lecithin solution | 300 µL |

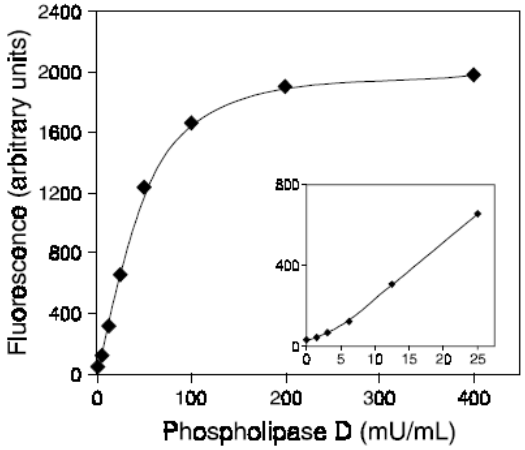


**Figure 1.** Detection of PLD using the Mes-Red reagent–based assay. Each reaction contained 50 µM Mes-Red reagent, 1 U/mL HRP, 0.1 U/mL choline oxidase, 0.25 mM lecithin and the indicated amount of *Streptomyces chromofuscus* PLD in 1X Reaction Buffer.Reactions were incubated at 37°C for one hour. Fluorescence was measured with a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm.

## Experimental Protocol

The following procedure is designed for use with a fluorescence multiwell plate scanner. For use with a standard fluorometer,volumes must be increased accordingly. Please note that the product of the Mes-Red reaction is unstable in the presence of thiols such as dithiothreitol (DTT) or 2-mercaptoethanol.For this reason, the final DTT or 2-mercaptoethanol concentration in the reaction should be less than 10 µM. The absorption and fluorescence of resorufin are pH-dependent. Below the $pK_a$ (~6.0), the absorption maximum shifts to ~480 nm and the fluorescence quantum yield is markedly lower. In addition, the Mes-Red reagent is unstable at high pH (>8.5). For these reasons, the reaction should be performed at pH 7–8. For assaying phospholipase D enzymes at moderately acidic pH, the reaction can be performed in two steps.

## Continuous PLD Assay (for PLD Enzymes with Near-Neutral pH Optima)

The following protocol describes the assay of PLD in a total volume of 200 µL per microplate well. The volumes recommended here are sufficient for ~100 assays.

**1.1** Dilute the PLD–containing samples in 1X Reaction Buffer. Use 1X Reaction Buffer without PLD as a negative control. A volume of 100 µL will be used for each reaction.

**1.2** Prepare a positive control by diluting the 20 mM $H_2O_2$ working solution to 10 µM in 1X Reaction Buffer.

**1.3** *Optional:* Prepare another positive control by diluting the PLD stock solution into 1X Reaction Buffer.

**1.4** Pipet 100 µL of the diluted samples and controls into separate wells of a microplate.

**1.5** Prepare a working solution of 100 µM Mes- Red reagent containing 2 U/mL HRP, 0.2 U/mL choline oxidase and 0.5 mM lecithin by adding 100 µL of Mes- Red reagent stock solution, 100 µL of HRP stock solution, 100 µL of choline oxidase stock solution and 50 µL of the lecithin solution to 9.65 mL of 1X Reaction Buffer. Note that this solution may be milky in appearance due to the lecithin. This 10 mL volume is sufficient for ~100 assays. Final concentrations of each component will be twofold lower in the final reaction volume.

**1.6** Begin the reactions by adding 100 µL of the Mes-Red reagent/HRP/choline oxidase/lecithin working solution to each microplate well containing the samples and controls.

**1.7** Incubate the reactions for 30 minutes or longer at 37°C, protected from light. Because the assay is continuous (not terminated), fluorescence may be measured at multiple time points to follow the kinetics of the reactions.

**1.8** Measure the fluorescence in a fluorescence microplate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm (see Figure 1).

**1.9** For each point, correct for background fluorescence by subtracting the values derived from the no-PLD control.

## Two-Step PLD Assay (for PLD Enzymes with Acidic pH Optima)

Some PLD enzymes have acidic pH optima. To assay these enzymes, you may wish to perform a two-step assay in which the PLD reaction is performed at a lower pH, and then the pH is raised to allow detection with the Mes-Red reagent. The following protocol can be used as a guideline for performing a twostep assay. The volumes recommended here are sufficient for ~100 assays, using a final reaction volume of 200 µL per assay.

**2.1** Dilute the PLD–containing samples in the reaction buffer of your choice. Use reaction buffer without PLD as a negative control. A volume of 100 µL will be used for each reaction.

**2.2** Add 0.5 µL of the 100 mM lecithin solution to each sample or negative control.

**2.3** Incubate the first-step reactions at 37°C for the desired length of time (e.g., one hour).

**2.4** Prepare a positive control by diluting the 20 mM $H_2O_2$ working solution to 10 µM in 1X Reaction Buffer.

**2.5** *Optional:* While the reactions are incubating, prepare another positive control by diluting the PLD stock solution into 1X Reaction Buffer.

**2.6** Pipet 100 µL of the diluted controls into separate wells of a microplate.

**2.7** Add 0.5 µL of the 100 mM lecithin solution to each control.

**2.8** Prepare a working solution of 100 µM Mes-Red reagent containing 2 U/mL HRP and 0.2 U/mL choline oxidase by adding 100 µL of Mes-Red reagent stock solution 100 µL of HRP stock solution and 100 µL of choline oxidase stock solution to 9.7 mL of 1X Reaction Buffer. This 10 mL volume is sufficient for ~100 assays. Concentrations of each component will be twofold lower in the final reaction volume.

**2.9** Begin the second step reactions by adding 100 µL of the Mes-Red reagent/HRP/choline oxidase working solution to each microplate well containing the samples and controls.

**2.10** Incubate the reactions for 30 minutes or longer at 37°C, protected from light.

**2.11** Measure the fluorescence in a fluorescence microplate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm (see Figure 1).

**2.12** For each point, correct for background fluorescence by subtracting the values derived from the no-PLD control.

### Storage condition

-20°C & Protected from light. Shelf life: 6 months after receipt.

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