Signal Guard™ HRP Reaction Stopping Solution

Ordering Information:	*Storage Conditions:*
Product Number: 11020 (0.5 mL)	Keep at -20 °C Avoid exposure to light

Introduction

Horseradish Peroxidase (HRP) coupling reactions provide sensitive assays on hydrogen peroxide-generating enzyme systems. Fluorogenic HRP substrates, including Amplex® Red (Cat#11000, Amplite™ ADHP), Amplex® UltraRed and Amplite™ Red, are preferred to be used for enhancing assay sensitivities, resulting in a continuous fluorescence increase. However, for high throughput screening applications it is hard to interpret the data collected from multiple time points, and ensure that the timing of the standard and unknown sample measurements is also critical to get accurate results. It is necessary for running HRP reactions simultaneously and quenching at a specific reaction time. Traditionally, HRP reactions can be stopped with acid, but the decay of signal is also a common problem for fluorogenic HRP substrate products.

Our Signal Guard™ HRP reaction stopping solution provides a convenient tool for terminating fluorescence signal-generating HRP reaction at a user-determined time point, and also keep the fluorescence signal stable for as long as 5 hours. The Signal Guard™ HRP reaction stopping solution is optimized for the HRP-induced reactions in conjunction with Amplite™ ADHP (Amplex® Red), Amplite™ Red and Amplex®UltraRed fluorogenic substrates.

Assay Protocol for one 96-well plate

1. Make **1X Stop Reagent** by dilution HRP Reaction Stopping Solution 20 folds, e.g., adding 50 µL of Signal Guard™ HRP reaction stopping solution into 950 µL ddH_2O.

   Note: Prepare the working solutions freshly as needed, and avoid light.

2. At the desired stopping time point, add 20 µL of **1X Stop Reagent** solution per 100 µL volume in each microplate well.

   Note: For other reaction volumes, adjust the addition of 1X Stop Reagent proportionally (e.g. add 5 µ L to a 25 µ L reaction volume). The 1X stop reagent should be added to all wells, including any reagent controls without HRP. The time-dependent fluorescence signal increase will terminate immediately and the fluorescence signal level should remain stable for at least 5 hours.

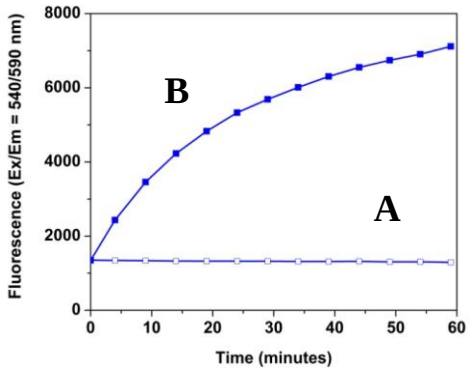

Figure 1. The Application of HRP Reaction Stop Solution in HRP-coupled glucose detection reaction. Two parallel reactions containing 15 µM Glucose were initiated by adding 50 µL assay mixture containing: 0.5mU/mL HRP, Amplex® Red, and 0.5mU/ml Glucose Oxidase. Reactions were incubated at room temperature for 5 mins and then 20 µL 1X Stop Reagent was added to one reaction (A), and 20 µL ddH_2O to the other reaction (B). The plots demonstrated that the reaction is completely inhibited by Signal Guard™ HRP Reaction Stopping Solution.