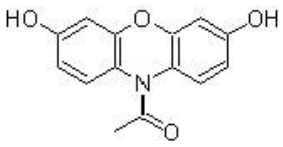
SuperRed, High Sensitivity ADHP Solution for ELISA with HRP Conjugates

Catalog No.:

TRED-80: Optimized for quantitative analysis, where titer, antigen concentration, ED50 or IC50 values are being measured.

TRED-LB-80: Optimized for the cut-off assay, where the signal-to-background (S/B) ratio needs to be maximized. The specialized formulation has low background, which enables overnight incubation to maximize the difference between the low antigen end of the curve from the blank (or negative control) in the assay.

Description:



10-Acetyl-3,7-dihydroxyphenoxazine (ADHP), is a sensitive and stable fluorogenic substrate for horseradish peroxidase (HRP). HRP is the widely used as a sensitive and stable enzyme in enzyme-linked immunosorbent assays (ELISAs). In the presence of HRP and H_2O_2, non-fluorescent ADHP generates highly fluorescent resorufin, which has maximum absorption at 571 nm and maximum emission at 585 nm.

Unlike other HRP substrates such as dihydrofluoresceins and dihydrorhodamines, ADHP shows minimal air-oxidation during storage and experiments. Thus far, ADHP has been known as the most sensitive and stable fluorogenic probe for detecting HRP and H_2O_2. ADHP has been widely used to detect HRP in many quantitative analyses of hydrogen peroxidase or different peroxidases, as well as in ELISA applications (1-9).

Due to the high extinction coefficient (ε) at ~570 nm of resorufin, the oxidation product of ADHP, it can also be used in colorimetric measurements at 570 nm.

Active Ingredients:

10-Acetyl-3,7-dihydroxyphenoxazine and hydrogen peroxide in optimized media

Instructions:

1. Before use, transfer the entire ADHP concentrated solution (400 µL) from the small vial into a brown bottle using a pipette and store it at 4°C. The product is only valid for 1 day after mixing.

2. For the best results, such as in quantitative analysis or when low background is desired, reconstitute and use the ADHP solution on the same day. For instance, if 20

mL of the solution is needed for that day, transfer 100 µL of the ADHP concentrated solution into 20 mL of diluent from the bottle (transferred from the original bottle to a different bottle/tube). Keep the newly reconstituted solution cooled over ice, and warm it to ambient temperature prior to use in the assay. 15 mL or 50 mL Falcon tubes can be used for this purpose. However, wrapping with aluminum foil is suggested to minimize light exposure.

3. The ELISA assay can be performed according to the standard protocol. It is recommended to perform an additional 1-3 wash cycles for the last wash step to wash off any unbound HRP conjugates.

4. Add 100 µL of reconstituted ADHP solution to each well and wait 5-60 minutes for TRED-80 and 0.5-20 hours for TRED-LB-80. The pink color should develop over time. Protect the solution from light until ready to read the fluorescence.

5. Read plates with a fluorescence plate reader with excitation at 550 nm and emission at 590 nm (or with a filter-based plate reader in similar wavelength ranges). If the signals are saturated with 550 nm excitation, one can set the excitation wavelength at 520-540 nm range. For colorimetric measurement, absorbance in each well can be determined at 570 nm.

Notes:

1. The optimized ADHP formulation is stable, but it is light-sensitive, so minimize the light exposure of the ADHP solution.

2. Avoid contact with metal surfaces or exposure to any solutions containing multi-valent metal ions since they will catalyze the non-enzymatic oxidation of ADHP by hydrogen peroxide or oxygen, resulting in a higher background in the assay.

3. Avoid direct pipetting from the bottle and transfer or pour out enough solution for a single use. Do not return any excess ADHP solution to the bottle to avoid potential contamination.