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High Intensity HRP-Chemiluminescence ELISA Substrate

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Description

The HaemoScan High Intensity HRP-Chemiluminescence ELISA Substrate uses an enhanced chemiluminescence formula which results in high intensity luminescence signals, providing high sensitivity during ELISA. The emitted light (425 nm) can be measured using a luminometer in either black or white opaque microplates or tubes. The substrate is specific for peroxidases such as horseradish peroxidase (HRP). The maximum signal intensity of the substrate is achieved shortly after initiation of the reaction and persists over time (Figure 1). The substrate consists of two components, making it easy-to-use and stable at room temperature for at least six months.

Principle

Peroxidases such as horseradish peroxidase catalyze the oxidation of luminol to 3-aminophthalate in the presence of a catalyst such as perborate. This reaction is accompanied by the emission of light at 425 nm which can be measured with a luminometer. In contrast to colorimetric (chromogenic) substrates which produce a colored product that persists after the enzyme-substrate reaction has occurred, chemiluminescence substrates produce light during the enzyme-substrate reaction and can be measured immediately.

Precautions/Handling

- The substrate is intended for research use only.
- The components should not be used beyond their expiry.
- Do not combine reagents with different lot numbers.
- Chemicals and reagents have to be treated as hazardous waste according to biohazard safety guidelines or regulations. For information on hazardous substances included in the substrate please refer to the Material Safety Data Sheets, which are available upon request.
- Avoid exposure to sunlight and extreme temperatures.
- Never pipette directly from the bottle, pour out the required amount to a plastic reservoir. Do not return excess solution.

Contents

- Perborate Solution 50, 125 or 250 mL per bottle
- Luminol Solution 50, 125 or 250 mL per bottle

Example of a Microplate Test Procedure

1. Perform an ELISA in an opaque microplate using a HRP-conjugate for detection.
2. Mix equal parts of Perborate solution and Luminol solution to obtain the High Intensity HRP-Chemiluminescence ELISA Substrate Working Solution.
3. Add 50-150 µL of the substrate per well and mix on a plate shaker for 1 minute.

Note: Increase the Working Solution volume as needed for test-tube applications.

4. Using a Luminometer, measure (425 nm) the relative light units (RLU) within 5 minutes after adding the substrate to achieve maximum signal intensity (Figure 1). When using a Luminometer, the total light output can be measured for the maximum sensitivity (the peak emission is only given as a reference).

Note: A purple/pink product may form during the reaction.

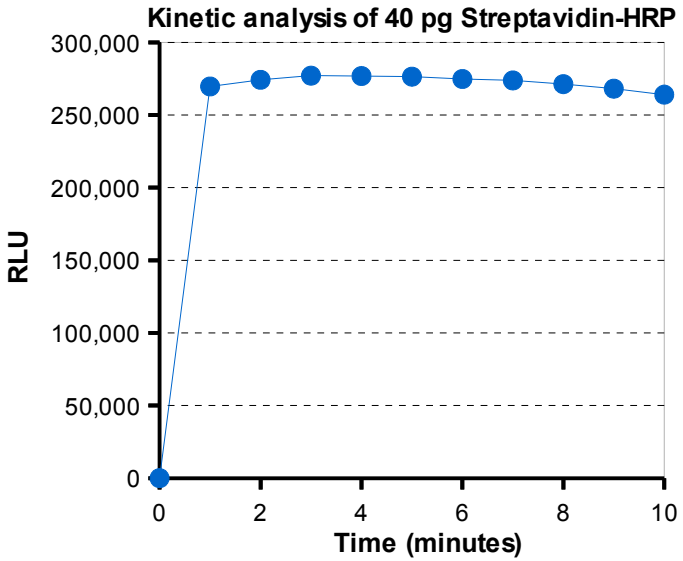

Figure 1. Rapid light generation with HaemoScan High Intensity HRP-Chemiluminescence ELISA Substrate. Streptavidin-HRP was diluted to 800 pg/mL in 0.1M Tris, pH 8.5 and 50 µL was transferred to a white opaque Nunc Maxisorp microplate. After addiction of 50 µL HaemoScan High intensity HRP-chemiluminecence ELISA substrate, the microplate was incubated at room temperature on a microplate shaker for 10 seconds and luminescence was measured with a gain of 120 at one minute intervals (GENios, Tecan Trading AG, Switzerland).