

# SuperSignal<sup>®</sup> ELISA Pico Chemiluminescent Substrate

37069 37070

0681.6

Number	Description
37069	<b>SuperSignal ELISA Pico Chemiluminescent Substrate</b> <b>Contents:</b> <b>SuperSignal ELISA Pico Luminol/Enhancer Solution, 125mL</b> <b>SuperSignal ELISA Pico Stable Peroxide Solution, 125mL</b>
37070	<b>SuperSignal ELISA Pico Chemiluminescent Substrate</b> <b>Contents:</b> <b>SuperSignal ELISA Pico Luminol/Enhancer Solution, 50mL</b> <b>SuperSignal ELISA Pico Stable Peroxide Solution, 50mL</b>

**Storage:** Upon receipt store product at room temperature. Product shipped at ambient temperature.

## Introduction

The Thermo Scientific SuperSignal ELISA Pico Chemiluminescent Substrate is for luminometer-based applications. This substrate is specific for peroxidase labels (e.g., horseradish peroxidase [HRP]) in ELISA procedures. The unique enhancer in this substrate results in rapid kinetic light output and high-signal intensity that lasts for up to 30 minutes (Figure 1).

## Important Product Information

- SuperSignal ELISA Pico Substrate is sensitive. Optimization of the antibody, antigen and HRP-conjugate concentrations is required.
- To limit nonspecific signal caused by cross-reactivity with antibody and the blocking reagent, empirical testing is essential when choosing an appropriate blocking buffer.
- To decrease background signal, a detergent, such as Tween<sup>®</sup>-20 (Thermo Scientific Surfact-Amps 20 Detergent Solution, Product No. 28320) at 0.05% final concentration, may be added to the appropriate blocking reagent.
- Do not use sodium azide as a preservative because azide is a known inhibitor of HRP.
- If a luminometer is not available for signal detection, X-ray film can be placed over an opaque microplate in a darkroom for 1-5 minutes. The film can then be processed by traditional methods.

## Example Microplate Procedure for Detection of Horseradish Peroxidase

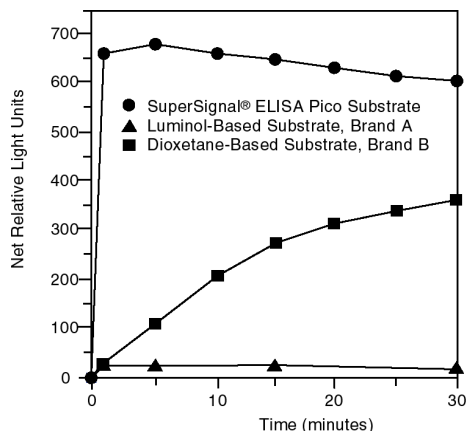
1. Using an opaque microplate, coat, block and wash the wells as required for an ELISA. Use a HRP-conjugate for detection.
2. Mix equal parts SuperSignal ELISA Pico Luminol/Enhancer and SuperSignal ELISA Pico Stable Peroxide Solution. This Working Solution is stable for approximately 24 hours at room temperature (RT).

**Note:** Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not harm the Working Solution.

3. Add 100-150 $\mu$ L of the Working Solution to each well. Agitate plate for 1 minute using a microplate mixer.

4. Use a luminometer to measure relative light units (~425nm) from 1 to 5 minutes after adding the substrate. Longer periods between adding the substrate and measuring the plate may result in decreased signal intensity.

**Note:** The peak emission wavelength is given for reference; however, for best sensitivity, measure total light output using a luminometer. Signal also can be measured in a test-tube luminometer. For test-tube applications, increase the Working Solution volume as needed.



**Figure 1.** Three chemiluminescent substrates were compared by adding 200pg of biotinylated HRP or biotinylated alkaline phosphatase to wells of Thermo Scientific NeutrAvidin Coated White Plates (Product No. 15116). Plates were incubated for 30 minutes at RT on a plate shaker and each well was washed three times with Tris-buffered saline (Product No. 28376). Substrates were prepared according to the manufacturer’s instructions. For SuperSignal ELISA Pico Substrate and the other luminol-based system (Brand A), 100µL of each substrate was added to the appropriate well. For the dioxetane-based system (Brand B), wells were washed with 1X assay buffer, and 100µL of substrate was added. All plates were incubated on a plate shaker at RT for 1 minute. Plates were measured on a Dynex MLX<sup>®</sup> Microplate Luminometer with a 0.2 second read time per well. Several measurements were performed during a 30-minute period.

## Related Thermo Scientific Products

- 15075 Reagent Reservoirs, 50mL, 40/pkg.
- 37538 StartingBlock™ (PBS) Blocking Buffer, 1L
- 37542 StartingBlock (TBS) Blocking Buffer, 1L
- 31030 High Sensitivity NeutrAvidin<sup>®</sup>-HRP, 0.5mL
- 21130 High Sensitivity Streptavidin-HRP, 0.5mL
- 21140 Pierce<sup>®</sup> Streptavidin Poly-HRP, 0.5mL
- 37075 SuperSignal ELISA Femto Maximum Sensitivity Substrate, 100mL
- 37515 SuperBlock<sup>®</sup> (PBS) Blocking Buffer, 1L
- 37535 SuperBlock (TBS) Blocking Buffer, 1L
- 37525 Blocker™ BSA in PBS (10X), 200mL
- 37520 Blocker BSA in TBS (10X), 200mL

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