

SuperSignal[®] ELISA Femto Maximum Sensitivity Substrate

37074 37075

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Number	Description
37074	SuperSignal ELISA Femto Maximum Sensitivity Substrate Contents: SuperSignal ELISA Femto Luminol/Enhancer Solution, 125mL SuperSignal ELISA Femto Stable Peroxide Solution, 125mL
37075	SuperSignal ELISA Femto Maximum Sensitivity Substrate Contents: SuperSignal ELISA Femto Luminol/Enhancer Solution, 50mL SuperSignal ELISA Femto Stable Peroxide Solution, 50mL

Storage: Stable for one year at 4°C or six months at room temperature. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific SuperSignal ELISA Femto Maximum Sensitivity Substrate is an enhanced chemiluminescent substrate developed for luminometer-based applications. This substrate is specific for peroxidase labels (e.g., horseradish peroxidase [HRP]) and can be used in ELISA procedures. The unique enhancer used in this substrate results in rapid kinetic light output and high signal intensity.

Important Product Information

- SuperSignal ELISA Femto Substrate is highly sensitive. Optimization of the antigen, antibody and HRP-conjugate concentrations may be required.
- To limit nonspecific signal caused by antibodies cross-reacting with the blocking reagent, choose an appropriate blocking buffer by empirical testing.
- To decrease background signal, a detergent, such as Tween[®]-20 (Product No. 28320) at 0.05% final concentration, may be added to the appropriate blocking reagent.
- Do not use 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.
- 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.

Example Microplate Procedure for Detection of Horseradish Peroxidase

A. Materials Required

- 96-well opaque microplate
- Capture antibody diluted to 5-10µg/mL in carbonate/bicarbonate buffer, pH 9.4 (Product No. 28382)
- Wash buffer such as Tris-buffered saline (e.g., 0.25M Tris, 0.15M NaCl; pH 7.2; Product No. 28376) or phosphate-buffered saline (e.g., 0.1M phosphate, 0.15M sodium chloride; pH 7.2; Product No. 28372)

- Blocking buffer such as Thermo Scientific StartingBlock (PBS) Blocking Buffer (Product No. 37538) with 0.05% Tween-20 or StartingBlock™ T20 (PBS) Blocking Buffer (Product No. 37539), which contains Tween-20
- Primary antibody at 0.05-0.1µg/mL
- HRP conjugate at 10-20ng/mL

B. Procedure

1. Use standard ELISA procedures to coat, block and wash microplate wells as required.¹
2. Mix equal parts of the SuperSignal ELISA Femto Luminol/Enhancer and SuperSignal ELISA Femto Stable Peroxide Solution. This Working Solution is stable for approximately 8 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 100-150µL Working Solution to each well.
4. Mix liquid in wells for 1 minute using a microplate mixer.
5. Use a luminometer to measure relative light units (~425nm) between 1-5 minutes after adding the substrate. Longer periods between adding the substrate and evaluating 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.

Related Thermo Scientific Products

15075	Reagent Reservoirs, 50mL, 40/pkg.
31030	High Sensitivity NeutrAvidin®-HRP, 0.5mL
21130	High Sensitivity Streptavidin-HRP, 0.5mL
21140	Pierce® Streptavidin Poly-HRP, 0.5mL
37069	SuperSignal ELISA Pico Chemiluminescent Substrate
37515	SuperBlock® (PBS) Blocking Buffer, 1L
37535	SuperBlock (TBS) Blocking Buffer, 1L
37525	Blocker™ BSA in PBS (10X), 200mL
37520	Blocker BSA in TBS (10X), 200mL

General Reference

1. Crowther, J. R. (1995). ELISA Theory and Practice: Humana Press. Totwa, NJ.

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Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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