

# Sensitive ELISA Substrate

## Description:

Sensitive ELISA Substrate is an enhanced and highly sensitive chemiluminescent HRP substrate optimized for chemiluminescent ELISA applications. This luminol-based substrate demonstrates a wide linear dynamic range and a superior signal to noise ratio for excellent quantitative ability. Sensitive ELISA Substrate allows for enhanced detection of low abundance proteins and reduced consumption of antibodies and other reagents.

- **Highest signal to noise ratio** – Superior performance
- **Broad linear dynamic range** – Enhanced detection and precision
- **Immediate light generation** – Fast detection
- **Cost effective** – Reduced reagent consumption

## For Orders:

Catalog Number	Product	Size
NE0516025A1	Sensitive ELISA Substrate	100 mL
NE0516025D1	Sensitive ELISA Substrate	250 mL

## Short Protocol:

1. Coat the ELISA plate with capture antibody diluted in 1X ELISA Coating Buffer and incubate 1h at room temperature (RT). General guidelines for capture antibody: use 0.01-1 µg/well.
2. Wash the plate with 1X Easy Wash Buffer 4-5 times (200-300 µL/well per wash).
3. Block the plate with 200 µL/well Easy ELISA Blocking Buffer and incubate 1h at RT.
4. Wash the plate with 1X Easy Wash Buffer 4-5 times (200-300 µL/well per wash).
5. Add the standards and samples (50-100 µL/well diluted in Easy ELISA Blocking Buffer) and incubate 1h at RT.
6. Wash the plate with 1X Easy Wash Buffer 4-5 times (200-300 µL/well per wash).
7. Add detection antibody diluted in Easy ELISA Blocking Buffer and incubate 1h at RT. General guideline for detection antibody: use 0.1-0.5 µg/mL and 50-100 µL/well.
8. Wash the plate with 1X Easy Wash Buffer 4-5 times (200-300 µL/well per wash).
9. Add HRP-conjugated antibody directed against the detection antibody diluted 1:10,000 to 1:50,000 in 1X Easy ELISA Blocking Buffer.
10. Wash the plate with 1X Easy Wash Buffer 4-5 times (200-300 µL/well per wash).
11. Mix Sensitive ELISA Substrate components 1:1 in sufficient amounts to obtain 9.6 mL of substrate solution mixture per each 96-well plate.
12. Use 100 µL of substrate solution (2 kit components mixed 1:1) per well to develop the plate. Measure the signal immediately after addition of substrate.
13. Read the plate on an ELISA microplate reader. Use settings for chemiluminescence detection recommended by the instrument manufacturer.