

## Description

A single step reagent for sub-picogram quantification of horseradish peroxidase in enzyme-linked immunosorbent assays and lectin-conjugate assays of glycobiology. Sensitive Blue™ reagent is designed to stabilize the blue chromophore, single oxidation product of 3,3',5,5'-tetramethyl-benzidine (TMB). This eliminates the necessity of assay development by the addition of a strong acid, stop reagent, allowing quantitative data to be obtained in real-time using a visible wavelength spectrophotometer or digital camera.

In the presence of peroxidase and H<sub>2</sub>O<sub>2</sub>, the aromatic amine TMB is converted to a blue chromophore, which has a peak absorbance at a wavelength of 370 or 650 nm. If desired, the blue TMB chromophore can be further oxidized with the addition of acid to form a yellow chromophore that has a peak absorbance at 450 nm.

1. Holland, V.R. *et al.* (1974) Tetrahedron 30:3299.

## What's Included

Sensitive Blue ELISA Reagent	250 mL
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**Storage:** Store all components at 4°C and away from light. Kit components are stable for at least six months.



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SENSITIVE BLUE ELISA REAGENT

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**CATALOG NUMBER**  
**FSB3005**

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## Reaction Procedure

1. Equilibrate Sensitive Blue ELISA Reagent to room temperature.
2. Add 50 - 100  $\mu$ L of Sensitive Blue ELISA Reagent to each microplate well that contains the peroxidase reaction.
3. Incubate the reaction for 15 to 30 minutes at room temperature.
4. Monitor real-time at 650 nm.

### Optional:

- Add 50 - 100  $\mu$ L of 2M sulfuric acid to each well to stop the reaction.
- Measure the absorbance of each well at 450 nm.

**Avoid contact with eyes, skin and clothing. Refer to MSDS for more information.  
MSDS available for download on our website**

## Additional Information

**Remember:** The Sensitive Blue ELISA Reagent can be oxidized by light (sunlight or UV) and various other oxidizing agents.

**For real-time analysis of blue chromophore -** Exclude optional steps of the procedure. However, readings will change over time.

### Potential Problems:

If a precipitate forms in the bottom of the wells, try reducing the peroxidase concentration or the incubation time.

If a color other than blue appears in the well, also try reducing the peroxidase concentration or the incubation time.

*For research use only. Not for use in humans.*