Fast TMB Substrate Solution, ELISA

CAT NO.: APL-1060

DESCRIPTION

Fast TMB Substrate Solution, ELISA is a one bottle stabilized chromogenic substrate for use with horseradish peroxidase immunoassays. It contains both 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in a one bottle format with long term stability and high sensitivity. It develops a deep blue color in the presence of peroxidase labeled conjugate, and is not applicable for use with assays requiring a precipitating substrate.

STORAGE

Product is light sensitive. Store in an amber bottle at 2-8℃. DO NOT FREEZE.

DIRECTIONS for USE

Fast TMB Substrate Solution, ELISA is a ready-to-use substrate. No mixing or additional reagents are required. This product does NOT need to be warmed to room temperature before use.

1. Thoroughly wash the microplate to remove all unbound enzyme conjugate.
2. Add the desired amount of Fast TMB Substrate Solution, ELISA to each assay well (100 µl -150 µl is recommended).
   Note: a multichannel pipette may be necessary.
3. Incubate the microplate at room temperature. Color will begin to develop immediately.
   Note: APOLO does not recommend diluting the substrate. Should the absorbance produced during the reaction be too high for your assay, you can:
   - Adjust incubation times
   - Adjust the concentrations or volumes of the other assay reagents
   - Try one of APOLO's lower activity TMB substrates

For Kinetic Assays

After the recommended incubation time, gently shake the microplate to evenly distribute the colored product. Measure the absorbance in the assay wells using a microplate reader set at a wavelength of 630 to 650 nm. The recommended wavelength is 650 nm. If measuring the absorbance using a dual wavelength mode, subtract the absorbance at 490 nm from the absorbance at 650 nm.

For Endpoint Assays

One of two stopping reagents should be used.

I. APOLO's Red Stop Solution which is a ready-to-use stop reagent designed so that the absorbance of the stopped reaction can be measured at 630 to 650 nm. Red Stop does not increase the background of the reaction and will help retain the absorbance of the reaction for at least 2 hours.
   Directions:

1. After the recommended incubation time, add 100 µl -150 µl of Red Stop Solution to each well. Gently shake the microplate to evenly distribute the colored product.
2. Measure the absorbance in the assay wells using a microplate reader set at a wavelength of 630 to 650 nm within 2 hours after the addition of the Red Stop Solution. The recommended wavelength is 650 nm. If measuring the absorbance using a dual wavelength, subtract the absorbance at 490 nm from the absorbance at 650 nm.

II. If using an acid stop solution, APOLO recommends 1N H_2SO_4.

Directions:

1. After the recommended incubation time, add 100-150 µl of acid to each assay well. The solution will turn yellow. Gently shake the microplate to evenly distribute the colored product.
2. Measure the absorbance in the assay wells using a microplate reader set at a wavelength of 450 nm within 2 hours after the addition of 1N H_2SO_4 (read within 30 minutes if not using 1N H_2SO_4). If measuring the absorbance using a dual wavelength mode, then subtract the absorbance at 650 nm from the absorbance at 450 nm.

PRODUCT USE LIMITATION

Research use only.