

TMB ELISA Substrate (Slowest Kinetic Rate) ab171527

3 Images

Overview

Product name	TMB ELISA Substrate (Slowest Kinetic Rate)
Tested applications	Suitable for: ELISA Unsuitable for: IHC-Fr or IHC-P
General notes	<p>Abcam's TMB ELISA Substrate (Slowest Kinetic Rate) contains 3,3',5,5'-tetramethylbenzidine in a mildly acidic buffer. The substrate is supplied as a ready to use solution. Unreacted substrate should be colorless or very light yellow in appearance. When this substrate system is reacted with peroxidase, a soluble blue reaction product is obtained. The reaction can be stopped using appropriate stop solution (see below), producing a soluble yellow or soluble blue reaction product, depending upon the stop reagent used, which is stable for at least 1 hour. ab171527 is not recommended for membrane or immunohistochemical applications that require a precipitating reaction product.</p> <p>Product Use:</p> <ol style="list-style-type: none">1. Allow substrate to equilibrate to room temperature (25°C) before use. Be sure to protect it from light during this process.2. After final antibody incubation and wash steps, tap the ELISA plate to remove excess liquid and add 100 µL of TMB substrate. The amount of substrate used should be tailored to your assay requirements.3. A soluble, blue color will slowly develop and can be read at 650 nm.4. Color development time can range between 10-40 minutes depending on the assay and will also depend on the method used to stop the reaction (see below). It is recommended that development time be optimized for your particular assay condition, however, a substrate color development time of less than 10 minutes is not recommended to ensure consistency of results.5. In endpoint assays, the substrate reaction can be stopped using equal volumes of 1 N HCl, 0.6 N sulfuric acid, or one of the Stop Solutions for TMB Substrates. (See 6a and 6b for O.D. recommendations)6. Stop Solutions have been optimized based on the desired endpoint.<ol style="list-style-type: none">a) The 450 nm Stop Solution is ab171529. They produce a soluble yellow product. Since stopping the reaction increases sample absorbance values approximately 2.5 fold, the maximum O.D. at 650 nm should be 1.2 to 1.3.b) The 650 nm Stop Solution is ab171531, which maintains the soluble blue color. Stopping with these reagents allows for the generation of O.D. values at 650 nm of up to 3.0, above which absorbance values are no longer linear with concentration. The 650 nm Stop Reagents are recommended for assays that need a large dynamic range without sacrificing detection limit.7. If the reaction color is too intense, it is recommended that you dilute either your antibody or conjugate. Dilution of the TMB substrate is NOT recommended.

Storage Instructions:

Exposure to direct sunlight and other UV sources should be avoided due to the light sensitive nature of the TMB molecule.

Properties

Form	Liquid
Storage instructions	Store at +4°C. Please see notes section.
Storage buffer	pH: 1.00 Constituents: 0.05% 3,3',5,5'-Tetramethylbenzidine, 79% Water, 0.1% Hydrogen peroxide

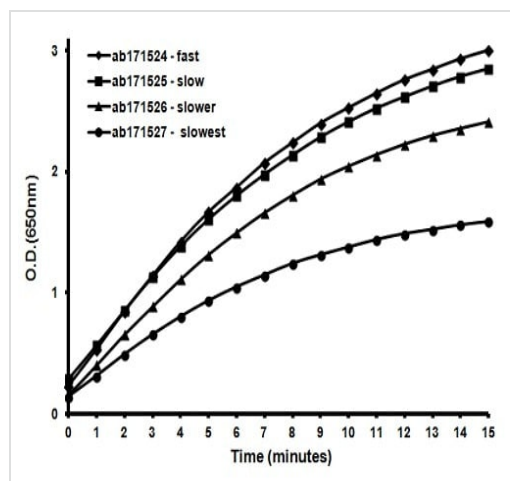
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab171527 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ELISA		1/1. 100 µL of substrate solution is added to each well.

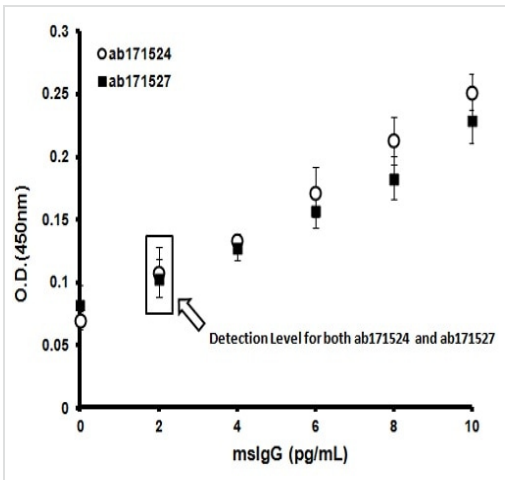
Application notes Is unsuitable for IHC-Fr or IHC-P.

Images



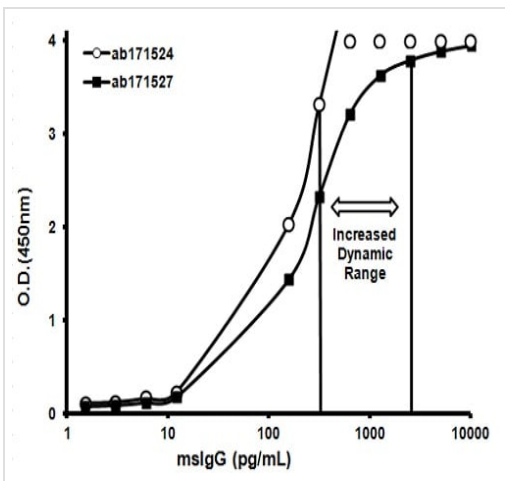
Comparison of the kinetic curves of several TMB substrates at 500 pg/mL mslgG monitored over time at 650 nm.

ELISA - TMB ELISA Substrate (Slowest Kinetic Rate) (ab171527)



Expanded graph at the lower concentrations using TMB ELISA Substrate (Fast Kinetic Rate) (**ab171524**) and TMB ELISA Substrate (Slowest Kinetic Rate) (ab171527). Both **ab171524** and ab171527 have the same detection limit.

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Standard curve comparison for TMB ELISA Substrate (Fast Kinetic Rate) (**ab171524**) and TMB ELISA Substrate (Slowest Kinetic Rate) (ab171527) stopped and read at 450 nm. Results show an extended dynamic range when ab171527 is used.

ELISA - TMB ELISA Substrate (Slowest Kinetic Rate) (ab171527)

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