

# Prestained TMB PLUS2, Ready-to-use Substrate

# Certificate of Analysis

**Cat. no.:** 4445

**Lot. no.:**

**Date of manufacture:**

**Exp. Date:** (When stored at 4°C in an unopened bottle).

**Analysis:** The substrate is analyzed for consistent activity, background, colour and pH (only the procedure for activity and background is described in the COA).

Background: 100 µl substrate is pipetted into four wells of a standard microtiter plate, followed by addition of 100 µl 0.2 M sulphuric acid. The background absorbance is read at 450 nm.

Activity: The activity is determined in a 2-layer ELISA. First layer is a standard human serum dilution. Second layer is rabbit anti human IgG-HRP. 100 µl substrate is added to each well and incubated for 13 minutes. The reaction is stopped by addition of 100 µl 0.2 M sulphuric acid. The absorbance is read at 450 nm. The activity is compared to a standard TMB solution with known activity.

**Specifications:** Activity: 90 - 110%  
Background: Abs. < 0.025  
Colour: Clear pink solution, abs. 0,137 – 0.204 at 550nm  
pH: 3,50 – 3,90

**Results:** Activity test:  
Background:  
Colour:  
pH:

**Storage:** Short term storage: Ambient; protected from light.  
Long term storage: 4 °C; protected from light.

**Performed by:** Date: Sign.:

**Approved by:** Date: Sign.: