

## QuantiChrom™ HRP Detection Reagent (DTMB-200)

### Quantitative Colorimetric Peroxidase Determination at 450nm

#### DESCRIPTION

*HORSE RADISH PEROXIDASE (HRP)* is an enzyme commonly used when conjugated to analytes of interest. When combined with hydrogen peroxide and a dye, HRP can be used to colorimetrically quantify the analyte.

#### KIT CONTENTS (200 TESTS IN 96-WELL PLATES)

- 500  $\mu$ L Reagent A
- 20 mL Reagent B
- 25 mL Stop Reagent

**Storage conditions.** The kit is shipped at room temperature. Store kit at 2-8°C upon receiving. Shelf life: 6 months after receipt.

**Precautions:** Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information. Vortex and bring all reagents to room temperature prior to assay.

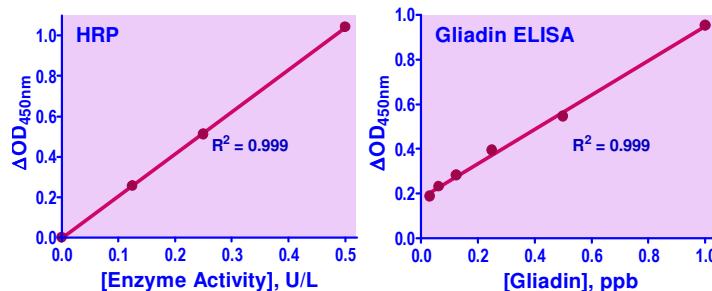
#### APPLICATIONS

Direct Assays of peroxidases, ELISA, immunochromogenic chemistry, Western blot

#### PROCEDURES

**Instructions for use:** Prior to assay, let reagents come to room temperature. Vortex reagents prior to test. For each well to be tested you will need 90  $\mu$ L Working Reagent, prepared by mixing 2.5  $\mu$ L reagent A and 100  $\mu$ L reagent B.

1. For ELISA, after washing off free HRP detection antibody, remove any remaining wash buffer. Add 90  $\mu$ L Working Reagent to each well.  
For a liquid sample, add 10  $\mu$ L sample to well. Add 90  $\mu$ L Working Reagent to each well.
2. Immediately tap plate to mix. Incubate 30 minutes at room temperature in the dark.
3. Add 120  $\mu$ L stop reagent to each well and read OD<sub>450nm</sub>.



Free HRP assay and Gliadin ELISA in 96-Well Plate using DTMB-200

#### DETECTION LIMIT

HRP:  $7.6 \times 10^{-8}$  U/well, or 7.7 pg/well;  
ELISA: 0.1 ppb Gliadin.

#### RELATED PRODUCTS

- QuantiFluo™ HRP Reagent (QFHRP-25mL), single-reagent, OD570nm, FL530/585nm.
- SuperLight™ HRP Reagent (SLHRP-100), single-reagent highly sensitive chemiluminescent reagent (to be released soon).
- QuantiFluo™ ALP Reagent (QFALP-6mL), single reagent for Alkaline Phosphatase detection, FL360/450nm.

#### LITERATURE

1. Li Y. et al (2018). NAD<sup>+</sup> Cofactor Regeneration by TMB - Mediated Horseradish - Peroxidase - Catalyzed Reactions. ChemistrySelect, 3(39), 10900-10904.
2. Busa L. S. A. et al (2016). 3, 3', 5, 5'-Tetramethylbenzidine oxidation on paper devices for horseradish peroxidase-based assays. Analytical Sciences, 32(8), 815-818.