

# ChromoSensor™ One Solution TMB Substrate

Technical Manual No. 0204

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## I. DESCRIPTION

Tetramethyl Benzidine (TMB), a noncarcinogenic analog of benzidine, is routinely used as the key substrate for enzyme immunoassays involving horseradish peroxidase-labelled (HRP-labelled) antibodies and molecular probes.

GenScript ChromoSensor™ One Solution TMB Substrate is a specially formulated, ready-to-use, ultra-sensitive, single-solution substrate for the detection of horseradish peroxidase (HRP) in Western blot and Dot blot assays.

## II. CONTENTS

We recommend 0.1 ml of substrate per cm<sup>2</sup> of membrane.

Component	L00222V60 For 600 cm <sup>2</sup>	L00222V125 For 1250 cm <sup>2</sup>	L00222V250 For 2500 cm <sup>2</sup>	L00222V 500 For 5000 cm <sup>2</sup>
TMB solution	60 ml	125 ml	2 x 125 ml	500 ml
Protocol	1	1	1	1

## III. KEY FEATURES

- Easy to perform: The substrate is delivered ready-to-use and requires no extra mixing.
- High sensitivity and low background: The substrate is optimized for a high signal and low background.
- Reproducible results: The kit produces highly reproducible results.

## IV. STORAGE

Store the kit at 2 to 8°C. It will remain stable for 12 months.

## V. PROTOCOL

This substrate is designed for the development of membranes from Western and Dot blots after the final wash step.

1. Drain off the excess wash solution from the membrane by holding it vertically with forceps and touching its edge against a tissue. Place the membrane on clean, flat surface, and cover it with the TMB solution (0.1 ml/cm<sup>2</sup>).
2. Incubate until the desired color is reached. Stop the reaction by rinsing the membrane three times for thirty seconds each in 20 ml of deionized water.
3. Drain off the excess water, and transfer the membrane to a piece of paper towel. Air-dry the membrane in a dark place.

## VI. EXAMPLES

### Western blot detection of housekeeping protein $\alpha$ -Tubulin

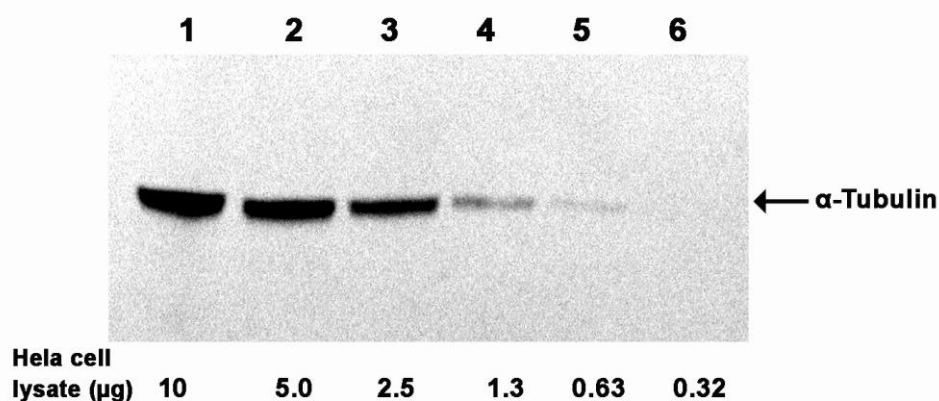


Figure 1. Western blot detection of  $\alpha$ -Tubulin using the ONE-HOUR Western<sup>TM</sup> Blot Kit (GenScript, L00205) and monoclonal  $\alpha$ -Tubulin antibody (abcam, ab7291). 10  $\mu$ g, 5.0  $\mu$ g, 2.5  $\mu$ g, 1.3  $\mu$ g, 0.63  $\mu$ g, and 0.32  $\mu$ g of HeLa cell lysate (BD Biosciences, 611449) were loaded in Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, and Lane 6, respectively. The blot was developed with ChromoSensor<sup>TM</sup> One-Solution TMB Substrate.

## VII. ORDERING INFORMATION

ChromoSensor<sup>TM</sup> Chemiluminescent HRP Substrate Kit:

Cat. No. L00222V60 Cat. No. L00222V125, Cat. No. L00222V250 Cat. No. L00222V500

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