



# K-Blue<sup>®</sup> Aqueous Substrate (TMB)

## Product Insert

100%  
Solvent-free

### Description

K-Blue Aqueous is a revolutionary 100% solvent-free, non-volatile TMB substrate. It contains 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in a one-bottle format with long-term stability and high activity. K-Blue Aqueous develops a deep blue color in the presence of peroxidase labeled conjugate and is ideal for end users working under strict regulatory requirements. This substrate is not applicable for use with assays requiring a precipitating substrate.

K-Blue Aqueous is compliant with Annex XVII for REACH and contains no hazardous substances at or above published reporting levels.

### Stability and Storage

K-Blue Aqueous is stable for 36 months when stored at 2-8°C.

### Appearance

Clear to light yellow-green solution

### Custom Packaging Service

Neogen can package K-Blue Aqueous in custom bottle sizes and volume fills to meet your specific packaging requirements. This service is a time and cost saving feature for any test kit manufacturer. For details on this service, please contact a Neogen Corporation representative.

Product #	Volume
331175	200 mL
331176	500 mL
331177	1 Liter
331257	20 Liters (1 x 20 Liters)

### Recommended Handling

TMB substrates are sensitive to certain handling and storage conditions. Please note the following precautions when handling Neogen's TMB substrates:

**Light Exposure** - TMB is very light sensitive and direct exposure to sunlight should be avoided. Prolonged exposure of the substrate to light (especially sunlight) should be avoided.

**Storage Containers** - The substrate should only be stored in high quality amber colored plastic or glass bottles. Neogen recommends HDPE amber bottles.

**Dispensing Precautions** - Some common metal ions (like iron) can oxidize TMB, causing an increase in background. Neogen recommends that only plastic or glass come in contact with the substrate. When using a dispensing pump make sure that no metal components of the pump come in contact with the substrate. Neogen further recommends that all pumps, tubing and storage containers be dedicated for use with K-Blue Aqueous ONLY. Neogen recommends platinum-cured silicone tubing.

Avoid the use of rubber stoppers and bottle caps containing rubber rings.

To avoid contaminating the entire bottle of substrate, never pipette directly from the substrate bottle. Always pour necessary volume of substrate into a separate container for use.

Do not leave the cap off of the storage bottle for prolonged periods of time.

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## Directions for Use

K-Blue Aqueous 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 K-Blue Aqueous substrate to each assay well (100  $\mu$ l -150  $\mu$ l is recommended). Note: a multichannel pipette may be necessary.
3. Incubate the microplate at room temperature. Color will begin to develop immediately.

**Note:** Neogen 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 Neogen'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. Neogen Corporation'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  $\mu$ l -150  $\mu$ l 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, Neogen recommends 1N H<sub>2</sub>SO<sub>4</sub>.

#### Directions:

1. After the recommended incubation time, add 100-150  $\mu$ l 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 30 minutes after the addition of the acid (if not using 1N HCl). If measuring the absorbance using a dual wavelength mode, then subtract the absorbance at 650 nm from the absorbance at 450 nm.

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## Technical Information

For technical support, please contact our Technical Service Department, Monday - Friday from 8:00 am - 6:00 pm EST.

Phone: 800/477-8201 (USA/CANADA)  
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## Warranty

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