Enhanced K-Blue® Substrate (TMB)
Product Insert

NO DMF OR DMSO

Description

Enhanced K-Blue is a one bottle stabilized chromogenic substrate for use with horseradish peroxidase immunoassays. It contains both 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in a one bottle format with long term stability and high sensitivity. Enhanced K-Blue develops a deep blue color in the presence of peroxidase labeled conjugate, and is not applicable for use with assays requiring a precipitating substrate. This formulation contains NO DMF or DMSO.

Sensitivity

Enhanced K-Blue is a highly sensitive substrate with kinetic reactions equivalent to Neogen's original K-Blue substrate formulation. This formulation offers the advantage of containing no DMF or DMSO and has very low background.

Stability and Storage

Enhanced K-Blue is stable for a minimum of 36 months when stored at 4°C.

Appearance

Clear to faint blue solution

Custom Packaging Service

Neogen can package Enhanced K-Blue 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.

Volume	Product #
200 mL	308175
500 mL	308176
1 Liter	308177
4 Liters	308199
20 Liters (1 x 20 Liters)	308257

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 Enhanced K-Blue ONLY. Neogen recommends Marprene® (UV resistant) 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

Enhanced K-Blue Substrate 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 Enhanced K-Blue Substrate to each assay well (100 µl -150 µl 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:

- Adjustincubationtimes
- Adjust the concentrations or volumes of the other assay re agents
- 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 µl -150 µl 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 HCl.

Directions:

1. After the recommended incubation time, add 100-150 µl 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.

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)
Phone: 859/254-1221 (International)
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Warranty

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the material from which its products are made are of standard quality. If any materials are defective, Neogen Corporation will provide a replacement product. Buyer assumes all risk and liability resulting from the use of this product and any of the predictive models. There is no warranty of merchantability of this product for any purpose. Neogen Corporation shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

Intended for Research and Manufacturing Use Only

 D308175-9-9/3/13