

KPL BluePhos[®] Microwell Phosphatase Substrate System

<u>Catalog No.</u>	<u>Size</u>
5120-0061 (50-88-02)	50 mL
5120-0059 (50-88-00)	600 mL
5120-0060 (50-88-01)	2700 mL

DESCRIPTION

This 2-component liquid substrate system develops a blue reaction product when reacted with phosphatase-labeled conjugates in microwell plates or tubes. This substrate is suitable for either qualitative or quantitative immunoassays. As a qualitative indicator, excellent visual discrimination can be achieved relative to substrates yielding orange or yellow reaction products. KPL BluePhos Phosphatase Microwell Substrate is not suitable for membrane or immunohistochemical staining.

Chemistry: 5-bromo-4 chloro-3-indolyl phosphate (BCIP) is converted by alkaline phosphatase to the enol 5-bromo-4-chloroindoxyl which, in turn, tautomerizes to its keto form. The keto compound undergoes an oxidation reaction to form 5,5'-dibromo-4-4'-dichloro-indigo white via an oxidation/reduction reaction with a tetrazolium compound. In the redox reaction the tetrazolium compound is reduced to the corresponding formazan. Using proprietary reagents, the formazan exists as a soluble blue chromophore with an absorbance maximum near 600 nm.

FORM/STORAGE/STABILITY

5120-0061 (50-88-02) consists of the following:
25 mL KPL Phosphatase Substrate Solution A
25 mL KPL BluePhos Microwell Phosphatase Solution B

5120-0059 (50-88-00) consists of the following:
3 x 100 mL KPL Phosphatase Substrate Solution A
3 x 100 mL KPL BluePhos Microwell Phosphatase Solution B

5120-0060 (50-88-01) consists of the following:
3 x 450 mL KPL Phosphatase Substrate Solution A
3 x 450 mL KPL BluePhos Microwell Phosphatase Solution B

Store components at 2-8°C. Stable for a minimum of three years from the date of manufacture when stored at 2-8°C.

CONTENT

The KPL BluePhos Microwell Phosphatase Substrate System contains soluble BCIP (5-bromo-4-chloro-3-indolyl-phosphate), at a concentration of 1 gram/Liter, and a proprietary tetrazolium.

USE

Preparation:

Warm to room temperature before use. Mix equal volumes of KPL Phosphatase Solution A and KPL BluePhos Microwell Phosphatase Solution B in a plastic or glass container immediately before use. Substrate will exhibit a clear, yellow color. Use within 30 minutes of mixing.

Substrate Development:

Following incubation with phosphatase (AP) labeled conjugate, wash plate thoroughly. Add 100 µL freshly mixed KPL BluePhos Microwell Substrate solution to each well. Tap gently to mix. Incubation times will vary depending on your assay.

ABSORBANCE MEASUREMENTS

Kinetic Assays:

KPL BluePhos Microwell Phosphatase Substrate produces a blue chromophore upon reaction with alkaline phosphatase. A measurement of $A_{620\text{nm}}/\text{min}$ (slope) vs. analyte concentration will yield appropriate standard curves. Read at a wavelength between 595 - 650 nm.

Endpoint Assays:

The addition of 100 µL of KPL APStop[™] Solution to the microwell plate will halt color development without shifting the absorbance maximum or substantially altering the absorbance value. A standard curve of absorbance vs. analyte concentration can then be constructed. Read at a wavelength between 595 - 650 nm. Stopped reaction should be read within 2 hours.

When to stop substrate reaction: The point at which the substrate reaction is stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate stopped before positive wells are no longer recordable. **For best results**, allow substrate to react for at least 10 minutes before stopping.

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PROCEDURE NOTES

- The dilution of the phosphatase (AP) conjugate must be optimized before using this substrate in a quantitative assay. If the conjugate concentration is too high, the linearity of the standard curve will be compromised.
- Users of pNPP substrate will have to dilute the phosphatase (AP) conjugate by a factor of 1.5 - 2.5.
- In contrast to peroxidase-catalyzed reactions, the phosphatase-catalyzed reaction of KPL BluePhos will remain linear for a **minimum** of 1 hour. Thus additional sensitivity (detection threshold) can be achieved by prolonged incubation with substrate.
- Care should be taken to avoid air bubbles when dispensing KPL BluePhos with a multi-channel pipette.

Substrate Reaction Too Fast?

To reduce the intensity of the substrate reaction, it is recommended that the conjugate and/or antibodies in the immunoassay be further diluted. Dilution of the substrate is not recommended. See PROCEDURE NOTES above.

PRODUCT SAFETY AND HANDLING

This product is considered non-hazardous as defined by the Hazard Communication Standard (29 CFR 1910.1200). Avoid contact with skin and eyes. In case of contact or spillage, clean with copious amounts of water. Product may be disposed via sanitary sewer.

U.S. Pat. No. 5,916,746 has been issued to KPL for a detection/signaling system based on formazan.

RELATED PRODUCTS

	CAT. NO.
KPL APstop™ Solution	5150-0026 (50-89-00)
KPL BSA Diluent/Blocking Solution	5140-0006 (50-61-00)
KPL Coating Solution	5150-0014 (50-84-00)
KPL AP Stabilizer	5290-0007 (55-15-00)
KPL pNPP Microwell Substrate	5120-0056 (50-80-00)
KPL PhosphaGLO AP Substrate	5430-0055 (55-60-04)
KPL PhosphaGLO Reserve AP Substrate	5430-0053 (55-60-02)

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.