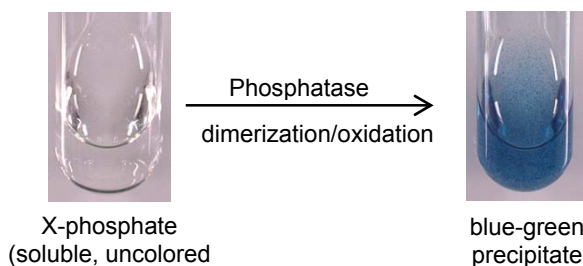


## 5-Bromo-4-chloro-3-indoxyl phosphate, disodium salt sesquihydrate

Cat. No. B-7450

### Introduction

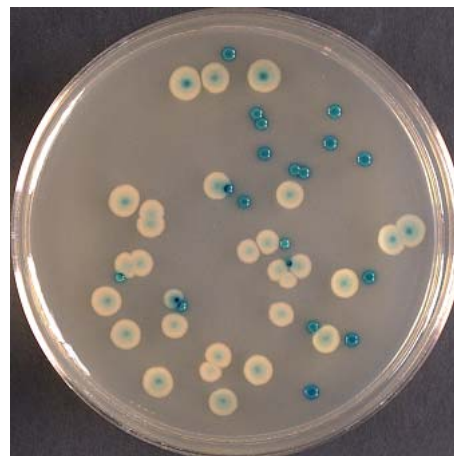
5-Bromo-4-chloro-3-indoxyl-phosphate (X-phosphate, X-Phos, BCIP) is a chromogenic indicator for phosphatase activity. The colorless product is cleaved by acid and alkaline phosphatases expressed by bacteria in liquid media or on agar plates and yields a blue-green colored precipitate (5,5'-Dibromo-4,4'-dichloro-indigo). X-phosphate can also be used as detecting agent for phosphatase coupled antibodies in Western blot and enzyme immune assay applications.



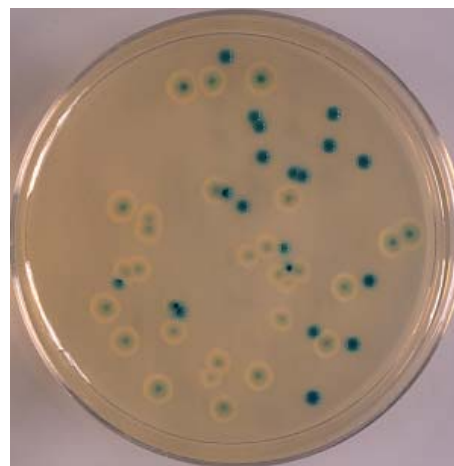
Color formation is due to dimerization and oxidation of 5-bromo-4-chloro-3-hydroxyindole which results from enzymatic cleavage of X-Phos. The reaction proceeds only in the presence of oxygen, i.e., in aerobic cultures. Well detectable coloration of bacterial colonies or liquid cultures is obtained within 24-48 h. In the presence of purified phosphatase, color develops within minutes.

### Application example

A mixture of *Staphylococcus aureus* (positive, blue-green color) and *Escherichia coli* (weakly positive) was plated on Nutrient Agar containing 0.25 mM X-phosphate. Plates were incubated at 37°C for 41 h.



Colonies of *S. aureus* (blue-green) and *E. coli* (white) on Nutrient Agar containing X-phosphate (picture was taken against a dark background).



(against a light background)

## Technical information

### Instructions for use:

Add 5-Bromo-4-chloro-3-indoxyl phosphate, disodium salt sesquihydrate (X-Phos) as filter-sterilized, concentrated stock solution after autoclaving.

### Solubility:

≥ 200 mM (159.0 mg/mL) in ultrapure water

≥ 25 mM (19.9 mg/mL) in 50% v/v ethanol

Recommended working concentration 0.1-0.3 mM (2-6 mL of 50 mM stock solution per L)

### Stability in growth media and aqueous solution:

X-Phos was stable for ≥ 3 weeks at 37°C in nutrient agar and in phosphate buffer pH 7.3.

### Reference strains:

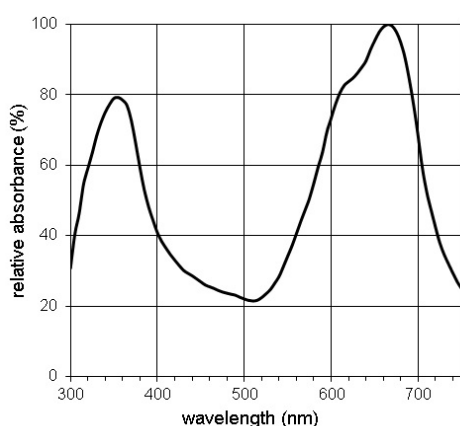
*Staphylococcus aureus* ATCC 25923, positive in aerobic culture

*Escherichia coli* ATCC 25922, weakly positive in aerobic culture

*Pseudomonas aeruginosa* ATCC 10145, negative in aerobic culture

### Absorbance spectrum:

50% v/v ethanol was added after enzymatic reaction for solubilization



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