

**Aldol® 515 L-proline amide, hydrochloride,**  
**Biosynth Patent: EP 2427431 and US**  
**8940909**

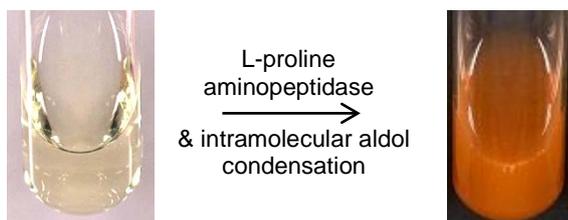
**Cat. No. A-4756\_P00**

M.W. 489.02 g/mol

## Introduction

Aldol® 515 L-proline amide, hydrochloride is a chromo- and fluorogenic indicator for L-proline aminopeptidase activity.

The colorless product is cleaved by L-proline aminopeptidase produced by microorganisms in liquid media or on agar plates and yields a red-orange color. In addition, red fluorescence signals are generated if a fluorescence enhancer or a suitable matrix is present. Aldol® 515 L-proline amide can also be used as detecting agent in enzyme assays.



Aldol® 515  
L-proline amide  
(uncolored)

red-orange  
color

Color and fluorescence formation is due to an intramolecular aldol condensation after enzymatic cleavage. The reaction proceeds equally well in the presence and absence of oxygen, i.e., in aerobic and anaerobic cultures. Well detectable color/fluorescence of bacterial colonies or liquid cultures is obtained within 16-48 h.

## Application example

Freshly grown *Hafnia alvei* RKI 75-3 (positive, *H.a.*) and *Escherichia coli* ATCC 25922 (negative, *E.c.*) were diluted in sterile saline, mixed and plated on Mueller-Hinton Agar containing 0.4 mM Aldol® 515 L-proline amide, hydrochloride. *H. alvei*, *E. coli*. and *Serratia marcescens* ATCC 7687 (negative, *S.m.*) were also streaked out on a second, similar plate. Plates were incubated at 37°C.

Coloration after 24 h

Mix *H.a.* + *E.c.*

top: *H.a.*

left: *S.m.* right: *E.c.*



## Instructions for use:

Add Aldol® 515 L-proline amide, hydrochloride as concentrated stock solution after autoclaving, stock solutions in organic solvents are auto-sterile.

### Solubility:

≥ 50 mM (24.5 mg/mL) in dimethyl formamide

### Recommended working concentration:

For coloration 0.2-0.4 mM (4-8 mL of 50 mM stock solution per L)

For fluorescence detection use a final concentration 0.05-0.1 mM of A-4756\_P00, with 0.3 mM of fluorescence enhancer A-4725\_P00

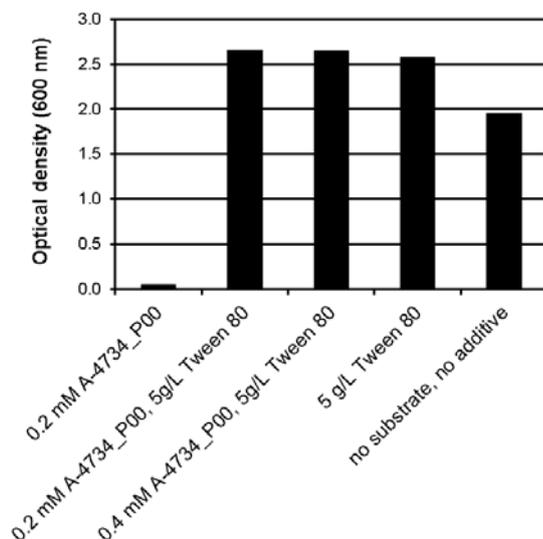
## Technical information

### Using Aldol® substrates for the detection of Gram positive bacteria

NOTE. Aldol® dyes show toxicity in certain strains of Gram positive bacteria.

Aldol® 515, 518, 484 and 495 substrates might inhibit the growth of aerobic and anaerobic Gram positive bacteria at the recommended working concentrations 0.2 - 0.4 mM.

No growth inhibition is observed in media supplemented with Tween® 80 in addition to Aldol® substrates. Tween® 80 can completely eliminate the inhibitory effect of Aldol®; however, antibiotics can interfere with this positive effect of the detergent.

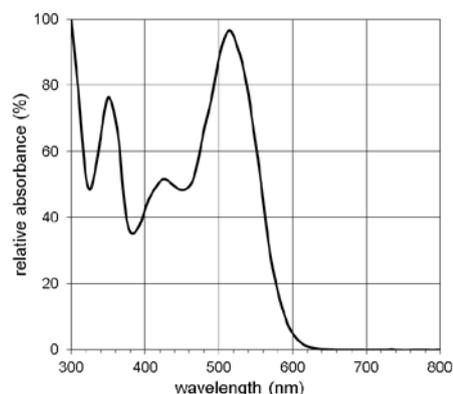


**Effect of Aldol® and Tween® 80** on the growth of Gram positive *Staphylococcus aureus* ATCC 25923. Optical density of broth cultures was measured after 22 h of incubation at 37°C and 150 rpm.

**CONCLUSION:** In any application aiming at differentiation of Gram positive bacteria with Aldol® substrates it is recommended to add 5 g/L Tween® 80 to the growth media. Compatibility of every target microorganism should be tested with Aldol® substrates and antibiotics in the growth media.

### Absorbance spectrum:

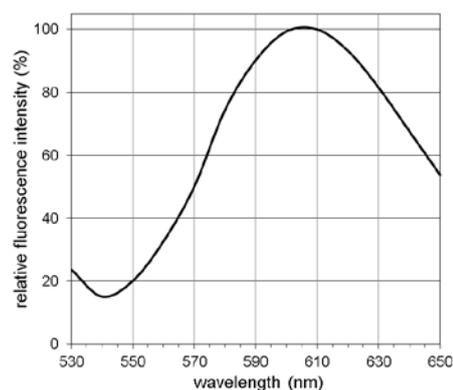
Aldol® 515 dye dissolved in ethanol



### Fluorescence emission spectrum:

Aldol® 515 dye dissolved in ethyl acetate.

Excitation: 500 nm



Fluorescence is obtained when bacteria are grown on cellulose acetate filters or in the presence of 0.3 mM Aldol® 355 fluorescence enhancer (Cat. No. A-4725\_P00). In liquid media, addition of 1% w/v Tween®80 also yields fluorescence.

Maximal fluorescence is obtained with excitation at 500 nm, excitation at other wavelengths between 330-420 and 470-490 nm is also possible.

### Reference strains:

*Hafnia alvei* RKI 75-3, positive in aerobic and anaerobic culture

*Escherichia coli* ATCC 25922, negative in aerobic and anaerobic culture

*Serratia marcescens* ATCC 7687, negative in aerobic culture