

# ProClin300 Preservatives

To solve the problem of too short shelf life of storing bio-specimen, ShineGene Co. has developed a new generation of high effective preservatives. These ProClin preservatives are highly effective biocides for controlling microorganisms in reagents and products intended for in vitro diagnostic use. Used at concentrations as low as 0.02%, these preservatives eradicate bacteria, fungi, and yeast for extended periods of time. Both preservatives offer excellent compatibility and stability with most enzyme systems, as well as low toxicity, so ProClin300 preservatives are an excellent choice for replacing thimerosal, sodium azide, and gentamicin preservatives. The price of this Product is fairly competitive and can be used by ELISA manufacturers in large batches.

## ● advantages of ProClin preservatives:

1. Broad-spectrum antimicrobial activity controls of bacteria, yeast, and fungi, and fast reaction speed.
2. Compatible with key enzymes, and do not inhibit antibody/ enzyme activity.
3. High effectiveness in low concentration. 6~20ppm ProClin300 can get a good preservative effect.
4. Effective at pH 2-8.5, and excellent stability. By Lab test, only 3% of the preservative contents will lose after 2 years of storage at 25°C.
5. Less toxic than thimerosal
6. Can be mixed with water at any ratio.

## ● The mechanism of Action of ProClin preservatives.

The active components in ProClin preservative are two isothiazolones: 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one. These active components have a unique mechanism of action that both inhibits microbe growth and causes cell death. Within minutes after contacting a microorganism, the active components penetrate the cell membrane and inhibit specific enzymes in the cell (figure 1). The target enzymes are within the central metabolic cycle of the cell, and ProClin preservatives aims at the 4 sites of the Krebs cycle (pyruvate dehydrogenase, a-ketoglutarate dehydrogenase, succinate dehydrogenase, NADH dehydrogenase) (figure 2), inhibiting metabolism, macromolecule synthesis, and causing intracellular energy levels to decline rapidly. With energy production disrupted, the cell can no longer synthesize chemicals for routine operation or repair. Ultimately the cell dies. Since most of the bacteria and fungi undergo at least part of the KREBS cycle, ProClin preservatives have a wide application scope. It is also proved that ProClin preservatives possess multiple pathways, which may reduce the likelihood of resistance development. With stopping of KREBS cycle, the cell's ability of generating energy (use ATP as an index, figure 3) and activity materials such enzymes has declined rapidly. But it will cause little harm to human or animals because of its low concentration usage.

图 2. ProClin 抑制位点示意图

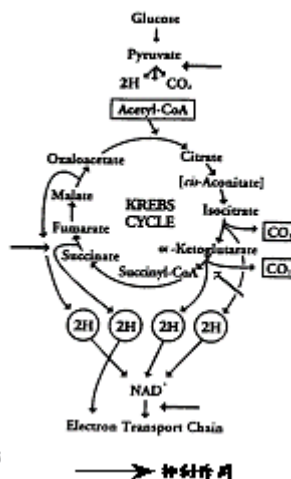


图 1. ProClin 抑制细胞生长

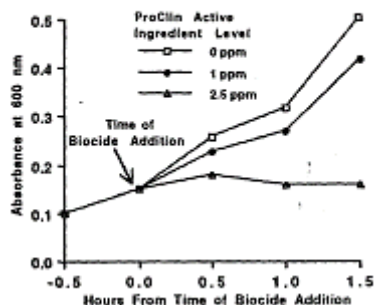
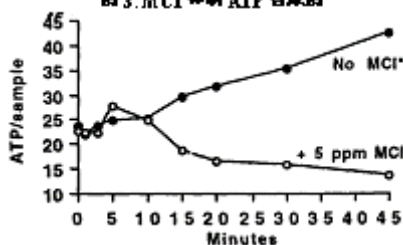


图 3. MCI 抑制 ATP 合成图

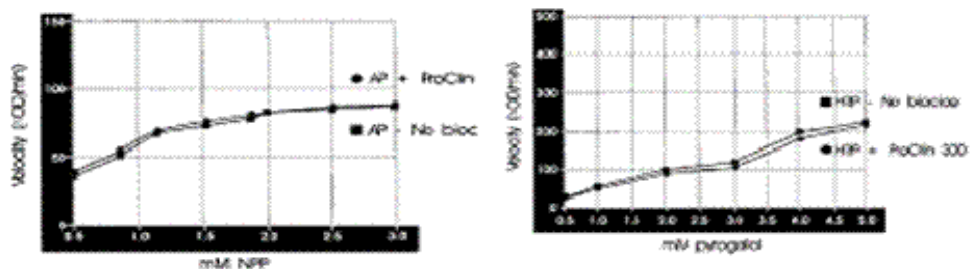


● study on ProClin preservatives side effect

1. ProClin preservatives influence on diagnostic reagents:

Lab test indicates that ProClin preservatives won't affect the binding speed of key enzymes and templates of diagnostic reagents, such as Horseradish Peroxidase (HRP) and alkaline phosphatase (AP)

Figure 4. 250ppm ProClin300 influence on binding speed of HRP(AP) with template



- short term effect

Even if ProClin300's concentration is 15 times higher than the recommended, the effect is little (table 1).

Table 1. ProClin300's influence on Vmax of HRP and AP

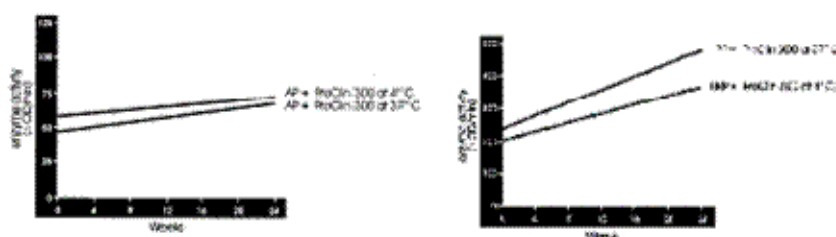
	Km (Mm)	Vmax (ΔA/min/mg protein)
HRP	6.9	904
+15ppm ProClin300	6.8	861

HRP	7.9	520
+250ppm ProClin300	8.3	525
AP	0.69	138
+15ppm ProClin300	0.56	131
AP	1.40	153
+250ppm ProClin300	1.20	142

### - Long term effect:

The long term effect on HRP and AP is also insignificant.

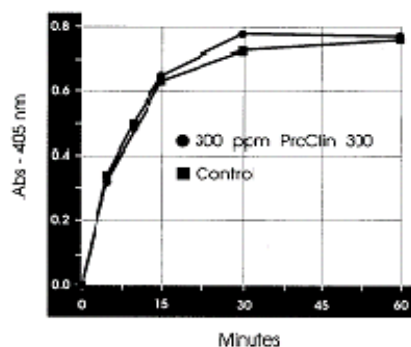
Figure 5. ProClin's effect on AP (HRP) activity at 4°C/ 37°C.



## 2. ProClin preservatives' influence on antibody binding

Used in recommended concentration, ProClin preservatives have little negative effect on antibody binding. After studying the ProClin 300's effect on binding of purified mouse IgM antigen and anti-mouse IgM antibody (binding HRP), it indicates that at a concentration level of below 300ppm, ProClin 300 won't affect the binding (figure 6). At 50ppm, ProClin 300 won't have a influence for 35 days.

Figure 6. ProClin300's influence on antibody of binding HRP.



## ● ProClin preservatives characteristics:

	Proclin 150	Proclin 200	ProClin 300	ProClin 5000
<b>Composition</b>				
Active Ingredients <sup>1</sup>	1.5%	1.5%	3.0%	50% <sup>2</sup>
Matrix	water	water	propylene glycol	dipropylene glycol
Stabilizer	23–25% Mg salts	3% Mg salts	alkyl carboxylate	none
<b>Recommended Level of Use</b>				

(as supplied,%)	0.04–0.10	0.04–0.10	0.02–0.05	0.01–0.03
Shelf Life	3 years	3 years	3 years	18 months <sup>3</sup>

#### Applications

in vitro diagnostic kits and kit components, calibration solutions, control solutions, organic reagents.

Only ProClin5000 is recommended for storing serum substrate.

<sup>1</sup> 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one

<sup>2</sup> 2-Methyl-4-isothiazolin-3-one only

<sup>3</sup> still collecting data.

### ● Final Concentration

This Product is liquid with concentration in use at 0.01%-0.05%.

### ● Storage

RT



## Shanghai ShineGene Molecular Bio-tech Co.,Ltd.

Add: Floor 2,Building A,328#, Wuhe Road, Shanghai,201109,China

Tel: +86-21-54460832

Fax:+86-21-54460831

E-mail:master@shinegene.org.cn

Website: [www.synthesisgene.com](http://www.synthesisgene.com)