



乙醛脱氢酶使用说明书

酶建议使用含有 0.02%牛血清白蛋白的 pH8.0 的 Tris HCl 缓冲液配制, 现配现用。反应体系中需要包含 0.1M 钾离子。以及 0.015M 的 β -巯基乙醇。以下为酶活检测方法, 体系仅供参考。

注意: 乙醛脱氢酶需要钾离子和巯基进行激活活性后发挥作用。



The amount of enzyme which will oxidize one micromole of acetaldehyde to acetic acid per minute at 25°C and pH 8.0 in the presence of potassium ions, thiols and β -NAD⁺.

1. 1.0 M Tris-HCl buffer, pH 8.0
2. 3.0 M KCl in distilled water
3. 0.005 M Acetaldehyde. Prepare fresh from stock 2.0 M Acetaldehyde.
4. 0.33 M 2-Mercaptoethanol. Prepared fresh.
5. 0.02 M β -NAD⁺ (15 mg/ml). Prepared fresh in distilled water.
6. Aldehyde dehydrogenase (enzyme) solution. Prepare in 0.1 M Tris-HCl, pH 8.0 containing 0.02% bovine serum albumin, to give a final concentration of 0.1-0.2 U/ml. Must be prepared fresh prior to assay.

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 340 nm and 25°C.

2. In a cuvette, pipette the following reagents in the amounts indicated:

Distilled water	2.20 ml	KCl	0.10 ml
Tris-HCl buffer	0.30 ml	α -2-Mercaptoethanol	0.10 ml
Acetaldehyde	0.10 ml	β -NAD ⁺	0.10 ml



3. Incubate cuvette in the spectrophotometer at 25°C for 5 minutes to attain temperature equilibration. Record absorbance at 340 nm (blank).
4. Initiate reaction by adding 0.1 ml enzyme solution to the cuvette. Record the increase in absorbance at 340 nm for 5 min.

Calculate E340nm/min

$$\text{Activity (U/mg)} = \frac{(\Delta E_{340\text{nm/min}})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

源叶