MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

Catalog Number: 190679
Aldehyde dehydrogenase potassium activated

Molecular Weight: ~200000
CAS # : 9028-86-8
Synonyms: E.C. 1.2.15; Aldehyde:NAD+ (P+)ov reductase
Physical Description: Off white to tan powder
Source: *Yeast*
Form: An off-white freeze-dried powder
Inhibitors: SH-reagents, such as N-ethylmaleimide, 2-iodobenzoic acid, iodoacetic acid and chloromercuribenzoic acid. Heavy metals (especially Cu^{2-}) and acetaldehyde (at high concentrations) inhibit. NAD, NADP, and acetaldehyde protect the enzyme from inhibition by the SH-reagents.
Activators: NH^{4-}, Pb^{-}, EDTA, histidine
Optimum pH: 9.0
pH Stability: 7.0
Thermal Stability: Below 40ºC
Specificity: The enzyme oxidizes a number of aliphatic and aromatic aldehydes. Acetyl-GSH is not hydrolyzed.
Assay Procedure:
The increase in absorbence is measured at 340 nm, Hg 334 nm or Hg 365 nm.
Reagents:

– Potassium pyrophosphate buffer (0.1 mol/L, pH 9.0): 3.3 g $K_4P_2O_7$, 15 mg dithiothreitol and 40 mg EDTA/~70 ml double distilled water; adjust with citric acid (2) to pH 9.0; adjust volume to 100 ml with double distilled water.
– Citric acid (1 mol/L): 2.1 g citric acid/10 ml double distilled water.
– NAD (0.03 mol/L): 20 mg NAD, free acid in 1 ml double distilled water.
– Pyrazole: 6.8 mg pyrazole/1 ml double distilled water.
– Acetaldehyde (~50 mmol/L): dilute 0.3 ml acetaldehyde with double distilled water to 100 ml (Caution: Do not pipette with mouth).
– Albumin (1%): 1 gm BSA/100 ml double distilled water.

Sample:
Volume activity should be ~0.20 to 0.25 units/ml.
Dissolve 20 mg lyophilizate in 1 ml double distilled water. Dilute 1:200 with ice cold albumin solution (6) immediately prior to measurement.
Wavelength:

340 nm: e340 = 6.3 L/(mmol X cm)
334 nm: e334 = 6.18 L/(mmol X cm)
365 nm: e365 = 3.4 L/(mmol X cm)

Light Path: 1 cm
Temperature: 25ºC
Total Volume: 2.91 ml
Sample Volume: 0.10 ml
Pipette into cuvette:

Buffer (1): 2.50 ml
NAD (3): 0.20 ml
pyrazole (4): 0.10 ml
sample: 0.10 ml

Mix, wait until ore-reaction stops (~2 minutes), incubate and check the temperature. Start reaction with acetaldehyde (5) 0.01 ml, mix, read the increase in absorbence (D, A) per minute using the linear portion of the curve.
Calculation:
One unit is the enzyme activity which reduces 1 umole of NAD per minute under the assay conditions (25ºC, pH 9.0).

Volume activity = (2.91/(e x 0.10 x 1)) x DA / minute [U/ml sample solution]
$Activity_{lyo}$ = (volume activity x 200)/20 [U/mg lyophilizate]

Remarks:

– Pyrazole is added for the inhibition of alcohol dehydrogenase.
– Acetaldehyde solution should be stored at +4ºC for at least two days before the assay (stable for at least 3 months).

Solubility: Dissolves readily at 5 mg/ml in 0.1M Tris/HCI pH 8.0 to give a clear colorless solution.
Unit Definition: That amount of enzyme causing the oxidation of one micormole of acetaldehyde to acetic acid per minute at pH 8.0 and 25°C, in the presence of potassium ions and thiols.
References:

– Steinmann, C.R., Jakoby, W.B., *J. Biol. Chem.*, **v. 242**, 5019 (1967).
– Clark, J.F., Jakoby, W.B., *J. Biol. Chem.*, **v. 245**, 6065 (1970).
– Black, S., *Methods in Enzymology*, **v. I**, 508 (1955).
– Steinmann, C.R., Jakoby, W.B., *J. Biol. Chem.*, **v. 243**, 730 (1968).
– Stoppani, A.O.M., Milstein, C., *Biochem. J.*, **v. 67**, 406 (1957).
– Jacobsen, M.K., Bernofsky, C., *Biochem. Biophys. Acta.*, **v. 350**, 277 (1974).
– Bradbury, S.L., Jakoby, W.B., *J. Biol. Chem.*, **v. 246**, 1834 (1971).
– Haeckel, R., Perlick, M, *J. Clin. Chem. Clin. Biochem.*, **v. 411** (1976).
– Haeckel, R., *J. Clin. Chem. Clin. Biochem.*, **v. 14**, 101 (1976)
– Bostian and Betts, *Biochem. J.*, **v. 173** (1978).