



Ethanol (K-ETOH) Procedure for ChemWell®-T Auto-Analyser

Requirements:

- Ethanol Assay Kit (K-ETOH) (provides ~ 600 assays).
- K-ETOH ChemWell®-T assay file and the K-ETOH % ChemWell®-T indices file.
- Use in association with the Ethanol Assay Kit (K-ETOH) product data booklet.

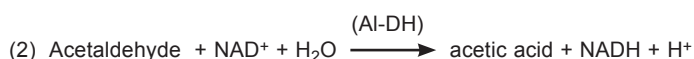
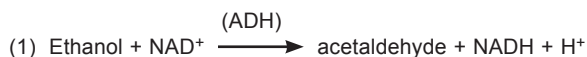
Use:

For the specific measurement of ethanol especially in wines, fruit juices, beverages and food products.

For specific sample preparation methods refer to the Ethanol Assay Kit (K-ETOH) data booklet.

Assay Principle:

Conversion of ethanol via the following reactions is directly proportional to the coupled formation of NADH:



Procedure:

Prepare the assay reagents and calibrators and use with the K-ETOH ChemWell®-T assay file. To generate values as % (v/v) use the K-ETOH % ChemWell®-T indices file.

Ethanol Assay Kit Components:

- Bottle 1:** Buffer (15 mL, pH 9.0) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.
- Bottle 2:** NAD⁺.
Stable for > 5 years at -20°C.
- Bottle 3:** Aldehyde dehydrogenase solution, (3.25 mL).
Stable for > 2 years at -20°C.
- Bottle 4:** Alcohol dehydrogenase suspension (1.3 mL).
Stable for > 2 years at 4°C.
- Bottle 5:** Ethanol standard solution (5 mL, 5 mg/mL).
Stable in a well sealed container (as supplied) for > 2 years at 4°C.

Preparation of Kit Components:

- Use the contents of bottle 1 as supplied.
Stable for > 2 years at 4°C.
- Dissolve the contents of bottle 2 in 12.4 mL of distilled water.
Stable for > 1 year at 4°C or > 2 years at -20°C (to avoid repetitive freeze / thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).
- 3 & 4.** Use the contents of bottles 3 and 4 as supplied.
Swirl the bottle to mix contents before use.
Stable for > 2 years at 4°C (ADH) or -20°C (Al-DH).
- To prepare the K-ETOH 4 calibrator (0.1 g/L) dilute 0.5 mL of the contents of bottle 5 to 25 mL with distilled water. Store in a well sealed Duran® bottle. When diluted, this solution is stable for 2 days at 4°C.
Use serial dilutions of K-ETOH 4 in distilled water to prepare K-ETOH 2 and K-ETOH 3.

Preparation of Assay Reagents: (per ~ 131 assays)

Reagent 1:

Component	Volume
distilled water	20.85 mL
bottle 1 (buffer)	2.4 mL
*bottle 2 (NAD)	2.4 mL
bottle 3 (Al-DH)	0.6 mL
Total volume	26.25 mL

*after adding 12.4 mL of distilled water

Reagent 1 stability: > 2 days at 4°C

Reagent 2:

Component	Volume
distilled water	3.2 mL
bottle 4 (ADH)	0.25 mL
Total volume	3.45 mL

Reagent 2 stability: > 2 days at 4°C

Calibrators:

- K-ETOH 1: 0 g/L (use distilled water)
K-ETOH 2: 0.025 g/L ethanol
K-ETOH 3: 0.05 g/L ethanol
K-ETOH 4: 0.1 g/L ethanol

Assay Parameters:

- Assay volumes: Reagent 1: 0.200 mL
Sample: 0.010 mL
Reagent 2: 0.025 mL
- Calibrators: 0, 0.025, 0.05, 0.1 g/L ethanol
Reaction time: 5 min at 37°C
Wavelength: 340 nm
Assay type: endpoint
Reaction direction: increase
Linearity: up to 0.1 g/L of ethanol

