BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

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Ethanol Assay Kit (Cat #: A-111)

COMPONENTS: Ethanol Assay Solution: 10 ml, store in aliquots at -20℃ or -80℃ after the first thawing (200 assays)
PEG Solution: 15 ml; store at 4℃

PRODUCT DESCRIPTION: Blood alcohol content (BAC) is most commonly used in medical and legal arenas. Serious intoxication can be observed with a BAC reading of 0.2%, and 0.4% is the accepted LD_{50} for adult humans. Being a central nervous system depressant, ethanol has profound psychoactive effects in sub-lethal doses. The assay kit takes advantage of the narrow specificity of yeast alcohol dehydrogenase (ADH), and thus primarily detects ethanol, although other straight chain primary alcohols and aldehydes also react with the assay solution. The kit is designed to measure ethanol in serum, urine, food beverages, and culture medium samples. Assay is based on the reduction of INT in a NADH-coupled reaction to formazan with a linear range of 0.01 - 0.2% ethanol. Since the assay reaction is enzymatic in nature, non-specific interference may occur with certain complex samples. The assay solution is stable for many years if stored in aliquots at -80℃.

PROTOCOL:

***Assay Solution:** Quickly thaw Ethanol Assay Solution, and keep solution on ice shielded from light during assay. Freeze the assay solution in small aliquots after the first thawing. Use -80℃ for long-term storage.

****Ethanol Standards:** Dilute absolute ethanol (not supplied) with dH_2O or a control medium to obtain 0.2%, 0.1%, 0.05%, and 0.025% ethanol standards.

*****Serum- or Plasma-containing Samples:** These samples need to be deproteinized by PEG precipitation prior to assay as follows. Mix 50 µl serum/plasma sample with 50 µl PEG Solution. Pipette PEG solution slowly due to viscosity of the solution. Vortex mixed solution vigorously and incubate on ice for 30 min. Clarify solution in a microfuge at maximal speed for 5 min. Transfer supernatant to another tube. The deproteinized sample is now ready for ethanol assay (see instructions below), or can be stored at -80°C.

******Urine and yeast culture medium:** Urine and yeast culture medium samples can generally be assayed directly without PEG treatment.

Assay:

1. Add 50 µl of the diluent (dH_2O or a control medium), ethanol standards, and samples to a 96-well microplate. Gently agitate thawed Ethanol Assay Solution before pipetting. Reaction is initiated by addition of 50 µl of Ethanol Assay Solution to each well. Mix contents by gentle but thorough agitation for 30 sec. Cover the plate, and incubate in a humidified 37°C incubator for 30 − 60 min. DO NOT use CO_2 incubator!

2. Stop reaction by adding 50 µl of 3% acetic acid (not provided) per well followed by gentle but thorough agitation for 30 sec. Eliminate any air bubbles present in the wells prior to measurement. Measure optical density at 492 nm using a microplate reader. Subtract the diluent reading from the standard and sample readings.

3. Generate a standard plot of ethanol concentrations vs. $O.D._{492nm}$ (see representative graph below). Apply sample readings to the standard curve to obtain sample ethanol concentration. A new ethanol standard plot must be generated for each assay. Multiply result by a factor of 2 for PEG-treated samples.

NOTES:

- Multiple freeze-thaw cycles should be avoided for the assay solution. The assay solution contains the organic solvent DMSO (9% v/v) and iodonitrotetrazolium violet (2 mg/ml). Please contact us or visit the product webpage for MSDS information.
- The PEG Solution and PEG-treated samples are viscous, and should be pipetted slowly and carefully to minimize assay errors.
- A 3% acetic acid solution needs to be prepared for reaction termination.