

BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Ethanol Assay Kit (Cat #: A-111)

COMPONENTS: Ethanol Assay Solution: 5 ml (100 wells), store at -80°C (**shield solution from light during use**)
PEG Solution: 3 ml, store at room temperature (**agitate vial to redissolve contents upon arrival**)

PRODUCT DESCRIPTION: Blood alcohol content (BAC) is most used in medical and legal arenas. Serious intoxication can be observed with a BAC reading of 0.2%, and 0.4% is the accepted LD₅₀ for adult humans. 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 may 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. The Ethanol Assay Solution is stable for many years if stored in aliquots at -80°C.

PROTOCOL:

Ethanol Assay Solution: Quickly thaw the Ethanol Assay Solution (*do not over thaw*). Keep solution on ice and shielded from light during assay. Freeze the assay solution immediately after use.

Ethanol Standards: First dilute anhydrous ethanol (not supplied with the kit) with dH₂O (or a suitable control vehicle) to obtain a 0.2% ethanol standard, e.g., 2 µl ethanol mixed with 1 ml dH₂O or a control vehicle. Perform 1:1 serial dilution to obtain 0.1%, 0.05%, and 0.025% ethanol standards.

Serum- or Plasma-containing Samples: These samples are deproteinized by PEG precipitation. Mix 25 µl of each sample with 25 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Shake tube vigorously followed by vortexing to ensure thorough mixing. Keep tube on ice for 5 min. Centrifuge solution in a microfuge at ~14,000 rpm for 3 min. Harvest supernatant and store at -20°C.

Urine and yeast culture medium: Although urine and yeast culture medium samples can generally be assayed directly without PEG treatment, PEG precipitation may be performed to avoid potential assay interference.

ASSAY

1. Thaw Ethanol Assay Solution quickly and keep solution on ice and shielded from light during assay. *Do not over thaw the solution.*
2. Add 50 µl of each ethanol standard and sample to a plain (uncoated) 96-well microplate.
3. Gently agitate Ethanol Assay Solution prior to first pipetting. Reaction is initiated by addition of 50 µl Ethanol Assay Solution to each standard and sample well. Mix contents by gentle but thorough agitation for ~10 sec. Cover plate and incubate at 37°C for 30 – 60 min. *Do not use CO₂ incubator.*
4. Eliminate air bubbles present in wells if necessary. Measure O.D. at 492 nm using a plate reader.
5. Plot ethanol standards vs. O.D._{492 nm}. Generate a trend line equation on chart. Calculate sample ethanol concentration using the derived equation (x = sample ethanol concentration; y = sample O.D._{492 nm}). A new plot must be generated for each assay. Multiply sample ethanol concentration by the applicable dilution factor.

Additional Information:

- Ethanol is flammable. Handle with caution. Ethanol Assay Solution contains the organic solvent dimethyl sulfoxide (DMSO) and iodonitrotetrazolium chloride. Please contact us or visit the product webpage for material SDS information.
- Agitate the PEG Solution vial in warm water to re-dissolve contents upon arrival.

