

K-ETOH FAQs (01/09)

Q1. I am experiencing problems obtaining consistent / accurate results using the Ethanol Assay Kit (K-ETOH) – what should I do?

A. This can be a common observation when using K-ETOH, however this problem is very rarely caused by faulty kit components and most commonly caused by equipment or environmental factors. K-ETOH is an extremely sensitive assay so extremely small amounts of ethanol present on any equipment that comes in to contact with any of the assay reagents or ethanol absorbed from the atmosphere will affect the K-ETOH assay results.

Ethanol kits from all manufacturers are extremely sensitive, and thus certain considerations must be taken into account, especially by certain users, such as in wineries or other laboratories where large quantities of ethanol (or other primary alcohols) are present. However, if the following precautions are observed, accurate results can be achieved with ease:

1. Water Quality - the same water (as ethanol / alcohol free as possible of course) should be used for both the assays and sample dilutions. The best method is to test your source of deionised / distilled water carefully before the analysis session. For example, let the machine producing the water operate for a minute or so, and then test it. When the quality is acceptable (in terms of a very small (i.e. < 0.100) absorbance rise in the blank reaction), put approximately 2 litres or so (in one container, for example a 2 L Duran bottle) aside for the entire analysis session. It is important this bottle (along with all other reagents in fact) is kept airtight, to avoid ethanol being picked up from the air. This occurs very quickly, even in laboratories where ethanol is not used, so in a winery for example, such interference is very rapid and significant.

2. Contaminated Pipettes - great care should be taken not to use pipettes that were previously employed to perform dilutions (or other manipulations) of wine / concentrated ethanol / alcohol standards, for the assays themselves. The ethanol will go into the vapour phase inside the pipette, and then back into the assay. Thus one step dilution of samples such as wine is very advisable (i.e. 0.1 mL in 100 mL of water for a 1 in a 1000 dilution).

3. Plastic-ware Contamination - certain pipette tips and cuvettes contain trace amounts of alcohol (i.e. a higher primary alcohol, not ethanol itself) that can lead to elevated / inconsistent results. If this is suspected, all plastic-ware should be washed with deionised / distilled water and dried properly before the experiment. This removes any traces of primary alcohols from the plastic-ware (NOTE: all short-chain primary alcohols react in the system).

4. Blank Reaction - as the reagents themselves will continually pick ethanol / alcohols up from the atmosphere (regardless of the precautions taken), the blank reaction will tend to yield a higher final absorbance value (relative to water or air), as the analytical session progresses (or in the long-term as the age of the kit increases). It is thus very important to run a blank with every set of samples, rather than just one at the beginning or end of the analytical session. Also, as there is a greater chance of sporadic interference with this kit, it is highly recommended that two blank reactions are run, so as to avoid a single faulty blank reading affecting all the results.

5. Environment - there is seldom little that can be done regarding the location of a laboratory's spectrophotometer, being it a central piece of equipment. However, attempting to perform ethanol analyses where a large number of open wine samples, for example, are being manipulated / stored will cause interference. The reasons are related to the very large concentration of this analyte in these samples (i.e. ~ 100 mg/mL), the volatile nature of ethanol, and the increased sensitivity of the assay, as compared to other enzymatic test kits (i.e. two NADH molecules are produced for every ethanol molecule in the sample). The solution is obviously to minimise the potential for interference as much

as possible, but this will be very situational:

(i) Perform ethanol assays on Monday mornings, i.e. before the manipulation of large quantities of wine / alcohol commences in the laboratory.

(ii) Locate the spectrophotometer as far away as possible from where wine / alcohol samples are processed / stored.

(iii) Set-up ethanol assays away from the spectrophotometer, and use cuvette caps to isolate the reactions from the general laboratory environment. Regardless of the situation, the use of plastic (recyclable) cuvette caps is higher recommended.

(iv) Let the other members of the laboratory know you are going to perform ethanol analyses, so they can minimise their use of alcohols. This is very relevant, especially in labs that clean benches regularly with ethanol sprays!

Q2. Can perchloric acid be used to deproteinise / clarify samples prior to analysis using the Ethanol Assay Kit (K-ETOH)? If so, how should such an extraction be performed?

A. Yes. Perchloric acid extraction can be used in conjunction with this kit, and should be performed as follows:

WARNING: If you have not worked with perchloric acid before, you must consult your safety officer for advice. Also, depending on the nature of the samples, it may be possible to reduce the concentration of perchloric acid, to for example 0.3 M (i.e. in the case of plasma). It is thus very important to determine if this is possible for each type of sample used, in order to reduce the risk from working with concentrated perchloric acid.

Liquid samples:

1. Carefully add an equal volume of ice cold 3 M perchloric acid and homogenise / fully disperse the sample (as appropriate).
2. After 15 min incubation on ice (or in a refrigerator), centrifuge at 3000 x g for 15 min at 4°C.
3. Neutralise by the slow addition of 2 M KOH.
4. Incubate on ice (or in a refrigerator) until the potassium perchlorate has settled out by gravity (approximately 10 min), and then simply remove some of the clear supernatant and use directly in the assay.

Solid samples:

1. Accurately weigh approx. 5 g of homogenised sample into a beaker containing 20 mL of 1 M perchloric acid and very carefully homogenise with an Ultraturrax® (or equivalent) for 5 min.
2. Carefully add approx. 40 mL of distilled water and neutralise using 2 M KOH (using pH test strips for example). Quantitatively transfer the contents to a 100 mL volumetric flask and fill to the mark with distilled water. If a fat layer develops, make sure this is above the mark, and the aqueous layer is at the mark.
3. Incubate in a refrigerator for approx. 20 min to allow separation of fat and precipitation of potassium perchlorate.
4. Filter through Whatman No. 1 filter paper, discarding the first few mL of filtrate, and use directly in the assay.