Sample preparation and purification for enzymatic testing
For 20 samples

For *in vitro* use only
Store between +2 and +8°C

Principle

The Enzytec Sample Purifier kit (E2250) **can be** necessary as pre-analytical step, as example for the Oxalate determination in beer, **but is not mandatory**.

The sample purifier contains active charcoal, which is able to eliminate smell, colour and all reducing substances (incl. ascorbic acid up to a certain concentration). The actions are substantially two:

– chemical one, through the oxidative action of Oxygen trapped in pores between carbon atoms.
– physical-chemical one, in particular adsorbing and trapping substances who have organic characteristics.

For the Oxalate test procedure, follow the insert of the Enzytec™ Oxalic acid kit (cat. N° E2100).

Reagents

Reagent 1: **Sample Purifier,** 20 tubes (powder from active charcoal), ready to use.

Reagent 2: **Sample Diluent,** 1 x 10 ml (liquid, 5x conc.). Add 40 mL of distilled water to the diluent and mix gently until complete mixing. Stability: 3 months at 2-8°C.

Reagents 1 and 2 are stable at 2-8°C up to the expiry date shown on the package, if not contaminated during handling. After dilution, reagent 2 is stable 3 months at 2-8°C.

Let the reagents reach the working temperature before use. Mix kindly before use. Close immediately after handling. The reagents have to be used properly, to avoid contamination.

The reagents are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample collection and storage

Do not use the Enzytec sample purifier kit for the standard from the oxalic acid kit (E2100), or from any other kit. If the standard would be used with E2250, there is nothing else than the analyte itself (oxalic acid for example) and part of it will be trapped by the charcoal, resulting into low recoveries.

a.) Food samples

- Oxalic acid is stable at low pH (<3.3). If the pH is above 7.5, oxalate will go into CO_2 and will be lost. But increasing the pH allows increasing solubility of Ca-Oxalate. So, the sample preparation should be performed between pH 5.0 and 7.0 because it is high enough to keep Ca-Oxalate in solution, and low enough to protect oxalic acid.
- If there is no need to solubilise Ca-oxalate, adjust the sample pH to 2.9 – 3.1.
- In some cases, oxalic acid and its salts have to be released: adjust the sample to pH = 3.0 with HCl (1M), and boil the sample in a water bath for about 15-30 minutes. Oxalic acid is stable at low pH, and boiling is meant to force hydrolysis of Ca-Oxalate. It is not necessary to correct the pH after boiling, because the pH 3.0 fits exactly to the test conditions for the oxalic acid test.

b.) Other samples

- The samples are collected (24 hours) in a glass or plastic bottle containing 10 mL of conc. Hydrochloric acid. Record the volume in liters. Since these samples are acidified, oxalate is stable and the samples can be stored for **7 days** when refrigerated or frozen.
- Take fresh samples with amount of vitamin C lower than 16 mmol/L (concentrations above this level may affect the test results).

Sample preparation

1. Prepare sample diluent (REAGENT 2) as mentioned before.
2. Set up a series of empty tubes for samples and controls and label them properly.
3. Pipete 5 mL of samples or controls into their corresponding tube (volume can be reduced if 5 ml are not available).
4. Add 2.5 mL of diluted sample diluent (REAGENT 2), then 2.5 mL of distilled water into each previous tubes, then mix.
5. **IMPORTANT NOTE FOR OXALIC ACID**
   The pH must be adjusted between 5.0 and 7.0 with HCl (1N) or NaOH (1N):
   – The pH of the sample diluent buffer is 8.2 – 8.5
   – If the sample has been stored with hydrochloric acid (see above), the pH after step 4 will be around 5.0-7.0 and a correction is in most cases not necessary
   – If the sample has been prepared differently (but always below pH 7.0), adjust the sample diluent between pH 5.0 and 7.0 before using it on step 4, so that the pH of the sample never exceeds pH 7.0
6. Set up a series of sample purifier tubes (REAGENT 1) for samples and controls and label them properly.
7. Pipete 4 mL of each DILUTED samples and controls (see points 4 and 5) to proper labeled SAMPLE PURIFIER tubes (see point 6) and mix for about 5 minutes by intermittent mixing. We suggest a rotator mixer for mixing.
8. Centrifuge the tubes for 10/15 minutes at 3500 rpm (2600 x g) or filter on laboratory filter paper. Or, place 0,5 mL of solution after mixing into an ultrafilter (ex. Millipore Ultrafree-MC, cat. UFC30HV00) and centrifuge for 3 min at 4000 rpm
9. Pick-up the sample in the middle of the supernatant, not from the surface, and use it for testing (e.g. oxalatic acid test).
10. Remember in the calculation to multiply all the results by factor 2 (if the sample volume under point 3 is lower than 5 ml, calculate the dilution factor accordingly).
11. **The Oxalate Standard from the Enzytec™ Oxalic acid kit (E2100) does not require this sample preparation; it is ready-to-use for the analytical procedure.**

Note:

This kit can be used with other kits than Enzytec™ Oxalic acid E2100. In this case, it is necessary to adapt the protocol according to the sample used on one side, and to the test used on the other:

– adjust the sample volume under point 3 according to the concentration of the analyte in the sample
– adjust the pH under point 5 according to the enzymatic test that will be used
– perform spiking experiments and check that recovery = 95 - 105%.