Saccharide Removal Kit (DSRK2-500)

Removal of Interfering Saccharides in Ethanol Assays

DESCRIPTION

Saccharides such as glucose and sucrose are known to interfere with the QuantiChrom™ Ethanol Assay (catalog# DIET-500). BioAssay Systems has developed a rapid procedure for complete removal of saccharides by co-precipitation with alkaline cupric and calcium ions. The treatment takes as little as 5 min and removes >99.9% saccharide from a sample.

APPLICATIONS

Rapid removal of interfering saccharides (e.g. glucose, sucrose) from samples such as culture media.

KEY FEATURES

Convenient and high-throughput. The procedure involves addition of two reagents sequentially and centrifugation for 2 min and transfer of the supernatant.

KIT CONTENTS (500 treatments)

Reagent A: 25 mL **Reagent B: 25 mL**

Storage conditions. The kit is shipped at room temperature. Store reagents at room temperature. Shelf life of 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SACCHARIDE REMOVAL

1. Transfer 100 µL Samples into Eppendorf tubes.
2. Add 50 µL Reagent A to Sample tubes and mix.
3. Add 50 µL Reagent B to Sample tubes and mix. Precipitates form instantly.
4. Centrifuge for 2 min at 14000 rpm in a table top centrifuge.

   The sample was diluted 2-fold (n = 2). After the precipitation, the supernatant contains ≤ 0.1% saccharide (based on 2% initial saccharide concentration). The supernatant is ready for ethanol determination with DIET-500 assay kit.

ETHANOL DETERMINATION (DIET-500)

1. Prepare 600 µL 2% Premix by mixing 120 µL 10% Standard and 480 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	Vol (µL)	Ethanol (%)
1	150µL + 0µL	150	2.00
2	120µL + 30µL	150	1.60
3	90µL + 60µL	150	1.20
4	60µL + 90µL	150	0.80
5	45µL + 105µL	150	0.60
6	30µL + 120µL	150	0.40
7	15µL + 135µL	150	0.20
8	0µL + 150µL	150	0

   Transfer 100 µL standards and 100 µL treated Sample into separate wells of a clear 96-well plate.

2. Quickly add 100 µL of Reagent A to the standards and Sample wells. Tap plate to mix.
3. Read kinetics at OD580nm for 10min at 25°C.
4. Data analysis: Compute ΔOD by subtracting OD values of the blank value (#8) from Standards and Samples. Plot the standard curve and fit with saturation binding equation ΔOD = a x [Ethanol] / (b + [Ethanol]) to determine a and b. Ethanol concentration (%), corrected for the 2-fold sample dilution, is calculated as:

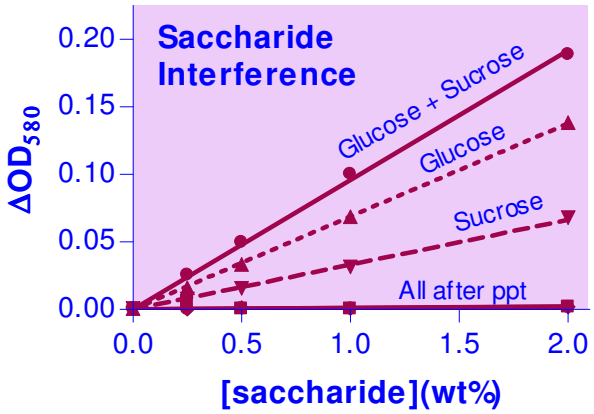
$$[\text{Ethanol}] = \Delta OD \times b / (a - \Delta OD) \times 2$$

SAMPLES TESTED

Fermented teas, wines, beer, culture media (e.g. YPD and DMEM), etc.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices. Eppendorf centrifuge tubes, table centrifuge and clear-bottom 96-well plates (e.g. Corning Costar).



Saccharide Interference in 96-well plate DIET-500 assay. Interference is eliminated upon precipitation of the saccharides using the DSKR2-500 kit.

Literature

1. Plimmer, RHA and Skelton, RF (1914). The Estimation of Allantoin in Urine in the Presence of Glucose. Biochem J. 8:641-8.

2. Pilone GJ (1985). Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: collaborative study. J Assoc Off Anal Chem. 68:188-190.

3. Dubowski KM (1980). Alcohol determination in the clinical laboratory. Am J Clin Pathol. 74:747-750.