Saccharide Removal Kit (DSRK-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.

APPLICATIONS

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 three reagents sequentially, incubation for 15 min, centrifugation for 5 min and transfer of the supernatant.

KIT CONTENTS (500 treatments)

Reagent A: 20 mL **Reagent B: 100 mL**
Reagent C: 20 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.

PROCEDURES

Procedure for use with DIET kit and 96-well plate:

1. Prepare 600 µL 2% Premix by mixing 120 µL 10% Standard and 480 µL distilled water. Dilute standard as follows. If possible when measuring ethanol in cell cultures, dilute the ethanol standard in media used. Transfer 100 µL standards and samples into eppendorf tubes.

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

2. Add 30 µL Reagent A to each tube and mix.
3. Add 150 µL Reagent B to each tube and mix.
4. Add 35 µL Reagent C to each tube and immediately vortex for several seconds.
5. Incubate 15 min at room temperature. Centrifuge for 5 min at 14000 rpm in a table top centrifuge.
6. Transfer 100 µL of each supernatant to a 96 well titer plate and proceed with the DIET-500 assay protocol (step2).

Procedure for use with the DIET kit and cuvette:

1. Prepare 2%, 1%, 0.5% standards and use distilled water as blank control. Transfer 300 µL diluted Standards and 300 µL samples to 1.5-mL eppendorf tubes.
2. Add 90 µL Reagent A to each tube and mix.
3. Add 450 µL Reagent B to each tube and mix.
4. Add 105 µL Reagent C to each tube and immediately vortex for several seconds.
5. Incubate 15 min at room temperature. Centrifuge for 5 min at 14000 rpm in a table top centrifuge.
6. Transfer 400 µL of each supernatant to a clean eppendorf tube and proceed with the DIET assay protocol (step2).

MATERIALS REQUIRED, BUT NOT PROVIDED

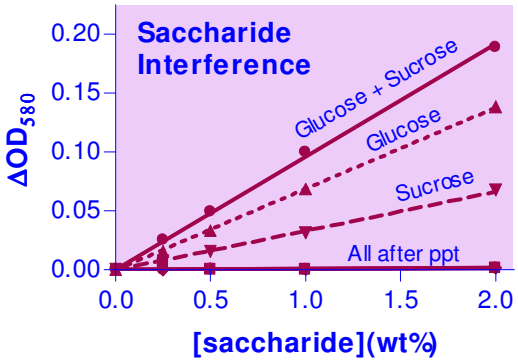
Pipeting devices.

Procedure using 96-well plate:

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

Procedure using cuvette:

Eppendorf centrifuge tubes, table centrifuge, cuvettes.



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