

User's Manual and Instructions

Saccharide Removal Kit (Z5030058)

Removal of Interfering Saccharides in Ethanol Assays

DESCRIPTION

Saccharides such as glucose and sucrose are known to interfere with the Ethanol Assay (catalog# Z5030029). BioChain 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 at least 6 months (see expiry dates on labels).

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 Z5030029 kit and 96-well plate:

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

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

- Add 30 μ L Reagent A to each tube and mix.
- Add 150 μ L Reagent B to each tube and mix.
- Add 35 μ L Reagent C to each tube and immediately vortex for several seconds.
- Incubate 15 min at room temperature. Centrifuge for 5 min at 14000 rpm in a table top centrifuge.
- Transfer 100 μ L of each supernatant to a 96 well titer plate and proceed with the Z5030029 assay protocol (step2).

Procedure for use with the Z5030029 kit and cuvette:

1. Prepare 2%, 1%, 0.5% standards and use distilled water as blank control. Transfer 300 μ L diluted Standards and 300 μ L samples to 1.5-mL eppendorf tubes.

- Add 90 μ L Reagent A to each tube and mix.
- Add 450 μ L Reagent B to each tube and mix.
- Add 105 μ L Reagent C to each tube and immediately vortex for several seconds.
- Incubate 15 min at room temperature. Centrifuge for 5 min at 14000 rpm in a table top centrifuge.
- Transfer 400 μ L of each supernatant to a clean eppendorf tube and proceed with the Z5030029 assay protocol (step2).

MATERIALS REQUIRED, BUT NOT PROVIDED

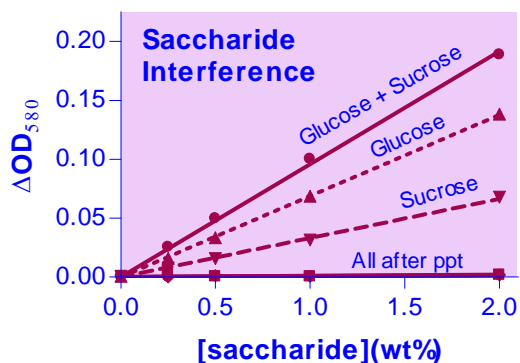
Pipeting devices.

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 Z5030029 assay. Interference is eliminated upon precipitation of the saccharides using the Z5030058 kit.

LITERATURE

- Plimmer, RHA and Skelton, RF (1914). The Estimation of Allantoin in Urine in the Presence of Glucose. *Biochem J.* 8:641-8.
- Pilone GJ (1985). Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: collaborative study. *J Assoc Off Anal Chem.* 68:188-190.
- Dubowski KM (1980). Alcohol determination in the clinical laboratory. *Am J Clin Pathol.* 74:747-750.