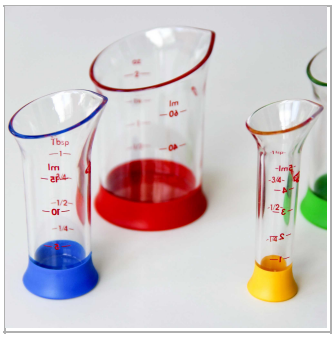

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We use this protocol and it's working

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Hestrin-Schramm (HS) medium

Andreas Sagen[1]

[1]University of Oslo

Andreas Sagen
University of Oslo, The National Institute of Occupational H...

ABSTRACT

Hestrin-Schramm medium is a common medium composition to grow *Komagataeibacter*. The medium contains glucose as a source for energy and carbon, while yeast extract and peptone are the primary nitrogen sources. Both sodium phosphate and magnesium sulphate is added to benefit the bacteria, but it is not necessary to support growth of *Komagataeibacter*. Citrate is used as an intermediate compound in the tricarboxylic acid cycle and an essential element to produce usable energy in aerobic organisms.

GUIDELINES

Follow step by step, unless stated otherwise. Equipment needed should be standard to a microbiology lab.

MATERIALS

Analytical scale, autoclave, bottle(s), weight vessel, LAF bench

SAFETY WARNINGS

You can mix Dextrose from the beginning with the other compounds, and autoclave together. While this is more time efficient and easier, it is important to take into account the possibility of toxic byproducts produced by the Millard reaction when autoclaving, producing Acrylamide, a probable human carcinogen (IARC Group 2A). Furthermore, when removing autoclaved components, be sure to take care as these can be very hot. If using antibiotics, use sufficient PPE to protect yourself, as some can be toxic to humans.

BEFORE START INSTRUCTIONS

Prepare glassware by cleaning it, and ensure that scale is sufficiently calibrated

1 All compounds are measured using a high precision analytical scale from powdered compounds. Each compound is measured to within 1% of the target weight. All compounds are mixed in a Duran bottle

100 mL Dextrose solution

1.1 Fill the bottle with 60 mL double-distilled water

1.2 Measure 10000 mg Dextrose

Powdered compounds:
Dextrose **Sigma-aldrich Catalog #G7021**

1.3 Add powdered solids into bottle, and use a magnetic mixer with a stir bar to mix for 00:05:00 5m

1.4 Adjust pH while mixing to pH 5.7 using concentrated sodium hydroxide

1.5 Add distilled water to a total of 100 mL

1.6 Autoclave liquid at 121 °C for 00:15:00 15m

2 All compounds are measured using a high precision analytical scale from powdered compounds. Each compound is measured to within 1% of the target weight. All compounds are mixed in a Duran bottle

500 mL HS (broth) medium

2.1 Fill the bottle with 300 mL double-distilled water

2.2 Measure 2500 mg Peptone, 2500 mg Yeast extract, 1350 mg Disodium phosphate, 750 mg Citrate and 600 mg Magnesium sulfate

Powdered compounds:
Peptone **Sigma-aldrich Catalog #P5905**
Yeast Extract **Sigma-aldrich Catalog #Y0875**
Disodium phosphate **Sigma-aldrich Catalog #S9763**
Citrate **Sigma-aldrich Catalog #S4641**

Magnesium sulfate **Sigma-aldrich Catalog #M7506**

2.3 Add powdered solids into bottle, and use a magnetic mixer with a stir bar to mix for 00:05:00 5m

2.4 Adjust pH while mixing to pH 5.7 using concentrated citric acid

2.5 Autoclave liquid at 121 °C for 00:15:00 15m

2.6 In a LAF bench, add 100 mL sterile Dextrose solution

2.7 Add sterile water to a total of 500 mL

> **Note**
>
> Cool to 50°C and supplement with antibiotics as appropriate

3 All compounds are measured using a high precision analytical scale from powdered compounds. Each compound is measured to within 1% of the target weight. All compounds are mixed in a Duran bottle

500 mL HS (agar) medium

3.1 Fill the bottle with 300 mL double-distilled water

3.2 Measure 2500 mg Peptone, 2500 mg Yeast extract, 1350 mg Disodium phosphate, 750 mg Citrate, 600 mg Magnesium sulfate and 7500 mg agar

Powdered compounds:

Peptone **Sigma-aldrich Catalog #P5905**
Yeast Extract **Sigma-aldrich Catalog #Y0875**
Disodium phosphate **Sigma-aldrich Catalog #S9763**
Citrate **Sigma-aldrich Catalog #S4641**
Magnesium sulfate **Sigma-aldrich Catalog #M7506**
Agar **Sigma-aldrich Catalog #A1296**

3.3 Add powdered solids into bottle, and use a magnetic mixer with a stir bar to mix for 00:05:00 5m

3.4 Adjust pH while mixing to pH 5.7 using concentrated citric acid

3.5 Autoclave liquid at 121 °C for 00:15:00 15m

3.6 In a LAF bench, add 100 mL sterile Dextrose solution

3.7 Add sterile water to a total of 500 mL

Note

Cool to 50°C and supplement with antibiotics as appropriate

Agar can be stored, then reheated to 50°C to be poured