**SOP Complex Gut Media preparation**

**Document Number: XX**

**Page 1 of 6**

**Purpose**

This SOP describes the procedure used to make Complex Gut Media.

**Scope**

For high diversity culturing from stool samples, it is a limited media that allows for small colony formation so fast and slow growing organisms can be grown on the same plate without the fast ones taking over

**Regulatory References:** NA

**Responsibility of experimentalist:** understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results.

**Responsibility of area manager or supervisor:** ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed, and updating the procedure as necessary.

**Definitions/Abbreviations**:

* Gram: g
* milliliter: mL
* microliter: uL
* Magnesium sulfate heptahydrate: MgSO4-7H2O
* Sodium bicarbonate: NaHCO3
* Sodium dichloride: NaCl2
* [Sodium hydroxide](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=0ahUKEwiWtuK0mo_MAhUDXD4KHTcoDD8QFggmMAE&url=https%3A%2F%2Fen.wikipedia.org%2Fwiki%2FSodium_hydroxide&usg=AFQjCNHdAaxt5c1oJz672c7x2mnGq0i3GA&sig2=KS5CFvpdojo_4_WN7N7gDQ): NaOH
* [Hydrogen chloride](https://en.wikipedia.org/wiki/Hydrogen_chloride): HCl
* Iron sulfate: FeSO4
* Dibasic potassium phosphate: K2HPO4
* Potassium dihydrogen phosphate : KH2PO4
* Calcium chloride : CaCl2
* DiMethyl sulfoxide: DMSO

**Related Documents:** NA

**Required Equipment and Materials / Reagents**

* Magnetic stirring bar (VWR: 58948-138)
* Magnetic stirrer (VWR: 12620-994)
* Sterile 1L bottle
* Bottle-top vacuum filter system, pore size 0.2 μm- 1L (Sigma Aldrich: Cat# CLS430515)
* UltraPure Distilled Water (Invitrogen: Cat# 10977-015)
* Tryptone (Sigma Aldrich: Cat# T793)
* Peptone from casein (Sigma Aldrich: Cat# 70173)
* Yeast Extract (BD: Cat# BD 212750)
* D-glucose (Alfa Aesar: Cat# 14431-43-7)
* L-cysteine (Sigma Aldrich: Cat# C7532)
* D-(+)-Cellobiose (Sigma Aldrich: Cat# 22150)
* D-(+)-Maltose (Sigma Aldrich: Cat# M5885)
* D-(-)-Fructose (Sigma Aldrich: Cat# F3510)
* Meat Extract (Sigma Aldrich: Cat# 70164)
* MgSO4-7H2O (Sigma Aldrich: Cat# 63138)
* NaHCO3 (Sigma Aldrich: Cat#S5761)
* NaCl2 (Sigma Aldrich: Cat# S5886)
* Vitamin Mix (ATCC: MD-VS)
* Trace Mineral mix (ATCC: MD-TMS)
* Resazurin (Sigma Aldrich: Cat# 62758-13-8)
* K2HPO4 (Sigma Aldrich: Cat# 7758-11-4)
* KH2PO4 (Sigma Aldrich: Cat# 7778-77-0)
* CaCl2 dihydrate (Sigma Aldrich: Cat# 10035-04-8)
* Menadione (Sigma Aldrich: Cat# 58-27-5)
* DMSO (Sigma Aldrich: Cat# 67-68-5)
* FeSO4 (Sigma Aldrich: Cat# 7782-63-0)
* DL-Histidine monohydrochloride (Sigma Aldrich: Cat# 123333-71-1)
* Hematine (Sigma Aldrich: Cat# 15489-90-4 )
* NaOH (J-T-Baker: Cat# 5635-02)
* HCl
* Tween80 (Sigma Aldrich: Cat# P4780)
* Nobel Agar (BD: Cat# 214230)

**Acid liquid component (stored in acid cabinet)**

* Acetic Acid (Sigma Aldrich: Cat# 64-19-7)
* Isovaleric Acid (Sigma Aldrich: Cat# 129542)
* Propionic Acid (Sigma Aldrich: Cat# P1386)
* Butyric Acid (Sigma Aldrich: Cat# B103500)

**Precautions**

* Personal protection equipment including gloves and lab coat must be worn when executing this procedure
* All liquid reagents have to be added to the dry mixture in the Chemical hood
* CGM media expires 3 weeks after preparation - so bottle have to be labeled with date of manufacture
* DMSO is a Combustible liquid/Causes skin irritation/Causes serious eye irritation: Keep away from heat / sparks / open flames / hot surfaces - No smoking - Wear protective gloves / eye protection / face protection.

**Procedure**

**Ingredients to mix for 1L of 2x media**

1. **Mix all dry component in sterile 1L bottle**

|  |  |  |  |
| --- | --- | --- | --- |
| **Component** | **Amount** | **Final Concentration**  **(at 1x)** | **Location** |
| Tryptone (none Bacto) | 4g | 0.2% | Chemical room |
| Peptone from casein | 4g | 0.2% | Chemical room |
| Yeast Extract | 2g | 0.1% | Chemical room |
| D-glucose | 0.8g | 2.2mM | Chemical room |
| L-cysteine | 1g | 3.2mM | Chemical room |
| D-(+)-Cellobiose | 2g | 2.9mM(0.1%w/v) | Chemical room |
| D-(+)-Maltose | 2g | 2.8mM(0.1%w/v) | Chemical room |
| D-(-)-Fructose | 2g | 5.5mM(0.1%w/v) | Chemical room |
| Meat Extract | 10g | 0.5%w/v | Chemical room |
| MgSO4-7H2O | 0.005g | 0.008mM | Chemical room |
| NaHCO3 | 0.8g | 4.8mM | Chemical room |
| NaCl2 | 0.16g | 1.37mM | Chemical room |

* Add 736mL of MilliQ Water

Note 1: Remove ATCC vitamin mix from -20C so it can thaw

1. **Add Liquid components to dry and water mixture**

Note 2: All liquid reagents have to be added to the mixture in the Chemical hood (SOP?)

Note 3: Components in bold are from stock solutions

|  |  |  |  |
| --- | --- | --- | --- |
| **Phosphate Buffer (1M)** | 200mL | 100mM | On bench |
| **CaCl2** | 2mL | 0.8% | On bench |
| **Vitamin K (menadione, 1mg/mL in DMSO)** | 2mL | 5.8mM | On bench |
| **FeSO4**  **(1.6mg/mL, 4x)** | 0.5mL | 1.44mM | On bench |
| **Histidine Hematin Solution**  **(**1.2 mg hematin/mL in 0.2M histidine) | 2mL | 0.1% | 4C |
| **Tween80 (25%)** | 4mL | 0.05% |  |
| ATCC Vitamin Mix | 20mL | 1% | -20C |
| ATCC Trace Mineral mix | 20mL | 1% | 4C |
| Acetic Acid | 3.4mL | 30mM | Acid cabinet |
| Isovaleric Acid | 0.2mL | 1mM | Acid cabinet |
| Propionic Acid | 4mL | 8mM | Acid cabinet |
| Butyric Acid | 4mL | 4mM | Acid cabinet |
| **Resazurin** | 2mL | 4mM | Chemical room |

1. Mix media with stir bar and magnetic stirrer
2. Adjust mixture to ~pH7 with 1M NaOH
3. Filter-sterilize final media mixture with Bottle-top vacuum filter system

Note 4: If you need to store media please cover it in aluminum foil and put at 4°C

**Stock solutions**

1. **Phosphate Buffer (1L,1M, pH 7.2):**

|  |  |
| --- | --- |
| **Component** | **Amount (mL)** |
| K2HPO4 | 717 |
| KH2PO4 | 283 |

Note 1: This stock solution needs to be filter sterilized, with Bottle-top vacuum filter system, before it can be stored at room temperature.

1. **CaCl2 dihydrate:**

|  |  |
| --- | --- |
| **Component** | **Amount** |
| CaCl2 dihydrate | 0.8g |
| Millipore DI H2O | 100mL |

Note 2: This stock solution can be stored at room temperature

1. **VitaminK (menadione) (6mL):**

|  |  |
| --- | --- |
| **Component** | **Amount** |
| menadione | 0.006g |
| DMSO | 6mL |

Note 3: This stock solution is light sensitive and needs to be wrapped in foil and store at 4°C

1. **FeSO4 (10mL, 4x):**

|  |  |
| --- | --- |
| **Component** | **Amount** |
| FeSO4 | 0.016g |
| Millipore DI H2O | 10mL |

Note 4: This stock solution can be stored at room temperature

1. **Histidine Hematin Solution:**
2. 0.2M Histidine solution

|  |  |
| --- | --- |
| **Component** | **Amount** |
| DL-Histidine monohydrochloride (Cat# H7875) | 01.5515g |
| Millipore DI H2O | 45mL |

- Adjust pH to 8.0 with 1M NaOH

1. Hematine Solution

|  |  |
| --- | --- |
| **Component** | **Amount** |
| Hematine | 0.0602g |
| 1M NaOH | 800uL |
| \*\*Wait for hematine to dissolve, might require heat\*\* | |
| 1M HCl | 400uL |

- Add all of hematine solution (1.2mL) to 0.2M, Histidine solution (~50mL)

- Adjust pH to 8.0 with 1M NaOH

Note 5: This stock solution needs to be filter sterilized, with Bottle-top vacuum filter system, before it can be stored for 3 month at 4°C (bottle have to be labeled with date of manufacture)

1. **Tween80 (25%, 24mL):**

|  |  |
| --- | --- |
| **Component** | **Amount (mL)** |
| Tween80 | 6 |
| Millipore DI H2O | 18 |

Note 6: This stock solution can be stored at 4°C

1. **Resazurin (24mL, 2x):**

|  |  |
| --- | --- |
| **Component** | **Amount** |
| Resazurin | 0.012g |
| Millipore DI H2O | 24mL |

Note 7: This stock solution can be stored at 4°C

**Version History**:NA

**Worksheets**: NA

**Appendix**: NA