**subSHI-V1 Media Preparation Protocol**

1. Preparation of stock solutions:
   1. Hemin Solution - Combine in flask and heat to boiling. Prepare 10ml aliquots and store at -20°C in FREEZER #2. Cover stored tubes with aluminum foil.

|  |  |  |  |
| --- | --- | --- | --- |
| 50mg | Hemin | Sigma, 51280 | 4C FRIDGE #2 |
| 1.74g | K2HPO4 | Fisher, P288-500 | Chemicals Shelf D558 |
| 100mL | ddH2O | MilliQ | D558 |

* 1. NAM Solution - Dissolve NAM in ddH2O and filter sterilize. Prepare 0.5-1mL aliquots and store at -20°C in FREEZER #2.

|  |  |  |  |
| --- | --- | --- | --- |
| 100mg | N-Acetyl Muramic Acid | Sigma, A3008-100MG | 4C FRIDGE #2 |
| 10mL | ddH2O | MilliQ | D558 |

* 1. Vitamin K3(Menadione)-Prepare 5mg/ml stock solution of Vitamin K3 in 100% ethanol as follows and store at 4C in FRIDGE #2 (Good for 1 month). Vortex until dissolved. Cover stored tubes with aluminum foil.

Add 200ul of the prepared stock to 1L of SHI media before autoclaving to obtain a final concentration of 1mg/L.

|  |  |  |  |
| --- | --- | --- | --- |
| 250mg | Vitamin K3(Menadione) | Sigma, M5625-25G | Chemicals Shelf D558 |
| 50mL | 100% Ethanol | 2701 | Flammable Cabinet(D558) |

* 1. Urea concentrated stock solution – Prepare a 0.1g/mL stock solution of Urea in ddH2O. Vortex until dissolved.

Add 600μL of the prepared stock to 1L SHI media before autoclaving to obtain a final concentration of 0.06g/L

|  |  |  |  |
| --- | --- | --- | --- |
| 1g | Urea | Fisher, U15-3 | Chemicals Shelf D558 |
| 10mL | ddH2O | MilliQ | D558 |

* 1. Arginine concentrated stock solution – Prepare a 0.1g/mL stock solution of Arginine in MilliQ H2O (ddH2O). Vortex until dissolved.

Add 1.7mL of the prepared stock to 1L SHI media before autoclaving to obtain a final concentration of 0.17g/L

|  |  |  |  |
| --- | --- | --- | --- |
| 1g | Arginine | Fisher, BP370-100 | Chemicals Shelf D558 |
| 10mL | ddH2O | MilliQ | D558 |

* 1. Sucrose solution – Prepare a 500 mg/mL and a 100 mg/mL sucrose solution.

Prepare a 500 mg/mL sucrose solution as follows. Filter sterilize. Cover stored tubes with aluminum foil.

|  |  |  |  |
| --- | --- | --- | --- |
| 5g | Sucrose | Sigma, S7903-250g | Chemicals Shelf D558 |
| 10mL | ddH2O | MilliQ | D558 |

Prepare a 100 mg/mL sucrose solution as follows. Filter sterilize.

|  |  |  |  |
| --- | --- | --- | --- |
| 2mL | 500 mg/ml | See above | 4C FRIDGE #2 |
| 8mL | ddH2O | MilliQ | D558 |

1. Combine the following in a previously sterilized bottle for 1L Shi Media:

|  |  |  |  |
| --- | --- | --- | --- |
| 10g | Proteose Peptone | Fisher, BP1420-500 | Chemicals Shelf D558 |
| 5g | Trypticase Peptone/Tryptone | BDBacto,211705/Fischer, BP 1421-500 | Chemicals Shelf D558 |
| 5g | Yeast Extract | BD Bacto, 212750 | Chemicals Shelf D558 |
| 2.5g | KCl | Fisher, P217-500 | Chemicals Shelf D558 |
| 10mL | Hemin Solution | (see 1a) | -20C FREEZER #2 |
| 600µL | Urea (0.1g/mL soln) | (see 1d) | Chemicals Shelf D558 |
| 1.7mL | Arginine (0.1g/mL soln) | (see 1e) | Chemicals Shelf D558 |
| 2.5g | Mucin | Sigma, M1778-10G | 4C FRIDGE #2 |
| 200ul | Vitamin K3 | (See 1c) | 4C FRIDGE #2 |
| 990mL | ddH2O | MilliQ | D558 |

1. Add all the components and mix using a stir bar until mucin dissolves visibly.
2. Check the pH of the media and adjust to 7.2 with 1N NaOH or 1N HCl if required.
3. Autoclave at 121°C for 15min.
4. Let cool to room temperature and store at 4°C.

BEFORE USE: (adjust values to the amount of SHI to be used)

1. Add the following to 1L autoclaved mixture

|  |  |  |  |
| --- | --- | --- | --- |
| 50mL | Sheep’s Blood | Colorado Serum Co., 31122 | 4C FRIDGE #2 |
| 1mL | NAM Solution | (see 1b) | -20C FREEZER #2 |

1. Heat inactivate FBS (gibco A31604-01) at 56 degrees C for 30 minutes.

-For 1L SHI containing 10% FBS, remove 100mL SHI and add 100mL heat inactivated FBS.

1. For 0.1% sucrose, add 1mL of 100mg/mL sucrose per 100mL SHI

-For 1L SHI containing 0.1% sucrose, remove 10mL SHI and add 10mL 100mg/mL sucrose solution.

Example: To prepare 45mL subSHI v1

* Start with 45mL autoclaved SHI.
* Add 2.25mL sheep’s blood to SHI. Vortex and invert to mix.
* Add 45µL NAM. Vortex and invert to mix.
* Remove 450µL SHI, then add 450µL of 100mg/mL sucrose. Vortex and invert to mix.
* Remove 4.5mL SHI, then add 4.5mL heat inactivated FBS.

Cover prepared SHI with foil and store in fridge until use.